MAGNETORECEPTION AND ITS NEURAL BASIS IN SPINY LOBSTERS

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ABSTRACT

David A. Ernst: Magnetoreception and Its Neural Basis in Spiny Lobsters
(Under the direction of Kenneth J. Lohmann)

The research presented here investigates magnetoreception (the ability to detect Earth's magnetic field) and its underlying mechanisms in the Caribbean spiny lobster, *Panulirus argus*. Spiny lobsters are the only invertebrates known to detect and use directional and positional information from Earth's magnetic field. Despite decades of research, we still do not know how lobsters, or indeed any animals, are able to perceive magnetic fields. To shed light on this elusive sensory modality, I examine the behavioral and transcriptomic responses of lobsters to strong magnetic stimuli.

Behavioral studies revealed that lobsters actively avoid dens with a strong magnetic anomaly and that lobster size is a predictor of avoidance behavior. On average, lobsters that chose dens with the anomaly were significantly smaller than those that chose dens with a non-magnetic weight. These findings are consistent with magnetoreception in lobsters, suggest ontogenetic variation in the lobster's response to magnetic fields, and indicate that magnetic anomalies might affect the movements of lobsters and other animals in the natural environment.

In additional behavioral studies testing the 'magnetite hypothesis' of magnetoreception, lobsters were subjected to a strong magnetic pulse (a stimulus thought to disrupt magnetoreceptors based on permanently magnetic material, such as magnetite), and

their subsequent orientation was tested. In contrast to controls, lobsters exposed to the pulse displayed directed orientation, consistent with magnetite-based magnetoreception.

Finally, transcriptomic approaches were used to identify candidate genes associated with magnetoreception and to determine the effects of a magnetic pulse on the lobster central nervous system. Hundreds of genes were differentially expressed throughout the nervous system in response to the pulse, many of which were associated with iron regulation and the oxidative effects of free iron on cells. These findings are consistent with the hypothesis that iron-based magnetoreceptors in the lobster central nervous system are disrupted or damaged by pulse magnetization. Furthermore, genes linked to diverse biological functions that are likely not linked to magnetoreceptors showed altered expression, suggesting that the pulse treatment had a significant impact on neural physiology. Together, these findings provide novel and significant insights into the mechanisms underlying magnetoreception and the physiological effects of pulse magnetization.

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CHAPTER I

INTRODUCTION

Movement, in some form, is a necessity of life for most animals. Acquiring food, avoiding predation, locating mates, and finding suitable habitat all require mobility and are critical for survival. The scale of movements can vary considerably, but traversing only a few centimeters can be just as significant for one species as traveling hundreds of kilometers is for another. Nevertheless, few animals move randomly in space; thus it is typically important to direct movement to at least some degree for it to be maximally beneficial. Given the varied ecological settings and life histories that exist in different animal species, it is perhaps not surprising that no single method of orientation, guidance, or navigation is universally superior across all environments and conditions. Instead, animals have evolved diverse, and often awe-inspiring, mechanisms and strategies to guide their movements.

One of the most fascinating and elusive sensory modalities linked to animal orientation and navigation is magnetoreception, or the ability to detect and extract information from Earth's magnetic field. Using Earth's field is a robust orientation strategy, as the geomagnetic field is present at virtually every location on Earth and is unaffected by changing environmental variables (Skiles, 1985). The earth's field resembles a massive magnetic dipole, with geomagnetic field lines exiting the earth's southern hemisphere (at the magnetic north pole) and reentering the planet in the northern hemisphere (at the magnetic south pole; Fig. 1.1A). The angle at which the field lines intersect Earth's surface, also

known as the inclination angle (Fig. 1.1B), reaches 90° at the magnetic poles and is parallel to the earth's surface (i.e., 0°) at the magnetic equator. This characteristic pattern provides animals that can detect inclination angle with a potential way to approximate latitude (Lohmann and Lohmann, 1994). Earth's magnetic field also varies in intensity (Fig. 1.1B) across the surface of the globe, with stronger field intensities toward the magnetic poles and weaker fields near the magnetic equator. Because both inclination angle and intensity vary in a predictable fashion across Earth's surface, most geographical locations are characterized by unique combinations of these parameters (Lohmann et al., 2007). These attributes reveal Earth's field as a source of both directional (i.e., 'compass') and positional (i.e., 'map') information for any animal capable of perceiving it (Johnsen and Lohmann, 2005).

An impressively diverse group of species are capable of magnetoreception, ranging from relatively simple invertebrate models, such as *C. elegans* (Vidal-Gadea et al., 2015) and *Drosophila* (Gegear et al., 2008), to birds (Wiltschko and Wiltschko, 1972, 2003, 2005), turtles (Lohmann and Lohmann, 1994, 1996; Lohmann et al., 2004), and even mammals (Begall et al., 2014). Amongst these various taxa, crustaceans have played a critical role in our understanding of the magnetic sense (Lohmann and Ernst, 2014). Crustaceans comprise an exceptionally large and diverse group of animals, inhabiting a variety of different terrestrial and aquatic habitats (Martin and Davis, 2007; Ahyong et al., 2011; Schram, 2012). Of the known extant ~70,000 species, most are mobile and rely on environmental cues to orient their movements, whether they are simply traversing a short distance to return to a burrow after a foraging event (Hughes, 1966; Vannini and Cannicci, 1995; Zeil, 1998) or undertaking long-distance, offshore migrations (Herrnkind and Kanciruk, 1978; Adamczewska and Morris, 1998).

The first studies to find evidence for a magnetic compass sense in crustaceans took advantage of movements by a marine amphipod (*Talitrus saltator*) that inhabits Dutch beaches (Arendse, 1978, 1980; Arendse and Kruyswijk, 1981). These amphipods display orientation along the land-sea axis, tracking the tides to avoid desiccation. Researchers found that canceling the ambient magnetic field around the amphipods abolished this axial orientation behavior, and shifting the direction of the field elicited comparable shifts in the preferred axis. Further work with African amphipods (*Talorchestia martensii*) found that exposure to a null magnetic field also disrupted this species' axial movements, resulting in random orientation (Ugolini and Pardi, 1992) and that the magnetic sense is dominant in the absence of solar cues (Ugolini et al., 1999; Ugolini, 2001, 2002). Similar results were observed in the marine isopod *Idotea baltica*; moreover, these isopods could be trained to associate slope with a magnetic axis (Ugolini and Pezzani, 1995).

The Caribbean spiny lobster, *Panulirus argus*, has become a promising animal for the investigation of magnetic orientation and navigation behavior. In early work with these lobsters, Creaser and Travis (1950) investigated their ability to home based on displacements of up to 8 km from capture sites near Bermuda. Impressively, approximately 20% of displaced lobsters were recaptured at the original capture sites, including some that had been released in deep water. These findings indicated an unexpected and extraordinary homing capability in this species. Indeed, additional studies confirmed that homing is a common and necessary occurrence for these animals, as lobsters embark on extensive nocturnal foraging bouts, after which they return to their home area to take refuge in a den before sunrise (Herrnkind et al., 1975; Herrnkind and Redig, 1975).

At the onset of autumn storms, lobsters undertake remarkable mass migrations offshore (Kanciruk and Herrnkind, 1978; Herrnkind, 1980). During these migrations, lobsters form single-file queues (sometimes in excess of 65 individuals), with each lobster extending its antennae anteriorly to maintain contact with the tail of the lobster preceding it. Intriguingly, queues within the same region typically adopt similar migratory headings (Herrnkind et al., 1973), even in areas devoid of useful visual cues, suggesting the use of another cue for orientation. Subsequent research showed that lobsters can detect and use wave surge for orientation (Walton and Herrnkind, 1977; Nevitt et al., 1995), but even in the absence of reliable wave surge and visual cues, lobsters were able to establish and maintain bearings consistent with their migration (Herrnkind, 1970; Herrnkind and McLean, 1971), suggesting the use of additional orientation cues.

Given that numerous animals take advantage of Earth's magnetic field for orientation and navigation (Wiltschko and Wiltschko, 1995), researchers hypothesized that lobsters might use a 'magnetic sense' to guide their foraging and migratory excursions. While the results from initial attempts at testing this hypothesis in the field were not definitive due to the presence of other confounding sensory cues (Walton and Herrnkind, 1977), Lohmann (1985) obtained the first evidence for magnetic sensitivity in lobsters by successfully training lobsters to associate the north-south magnetic axis with a food reward.

Additional work using an undersea magnetic coil system provided even stronger evidence for magnetoreception in lobsters (Lohmann et al., 1995). In this study, lobsters were captured from a patch reef in the Florida Keys, fitted with eye caps to obstruct all visual cues, and tethered so that they could walk on a Plexiglas platform centered inside the coil system positioned on the seafloor. Lobsters were allowed to establish a heading and were then

subjected to one of two treatments: either no change in the magnetic field (controls) or a reversal of the horizontal component of Earth's field (i.e., the direction of north within the coil was rotated to point toward magnetic south). The lobsters that did not experience a reversal maintained their initial headings, but lobsters exposed to the reversed field deviated significantly from their initial course, eventually adopting courses approximately opposite in direction to their original ones. Furthermore, lobsters subjected to a treatment that inverted the vertical component of Earth's field (Fig. 1.1B), a stimulus designed to determine if a compass is sensitive inclination angle, did not elicit significant deviation in orientation. Taken together, these results indicate that lobsters possess a magnetic compass sense that is likely based on the polarity (i.e., direction of the horizontal component) rather than the inclination angle of the geomagnetic field.

Perhaps the most intriguing evidence for magnetoreception in lobsters comes from work investigating whether lobsters are capable of true navigation, or the assessment of position relative to a goal after displacement to a novel location without the use of information obtained during displacement or cues originating from the home location. In this set of experiments, Boles and Lohmann (2003) displaced lobsters 12-37 km from their capture site, while employing techniques to prevent access to inertial, visual, chemical, and magnetic cues during transport, and tested their orientation within a seawater-filled arena the following morning. Remarkably, lobsters were able to determine the direction leading back to the capture location, indicating that they could somehow assess their geographic position using only cues derived from the testing location. To explore if lobsters were using a magnetic map sense to accomplish this feat, Boles and Lohmann (2003) conducted further studies using a magnetic coil system to "magnetically displace" lobsters and observe their

subsequent orientation. In other words, lobsters were subjected to magnetic fields replicating the fields that exist in locations north and south of the capture location, but were not physically displaced. In each case, lobsters oriented in directions consistent with the direction of the capture site, providing strong evidence that they are able to derive positional information from Earth's magnetic field and use it to navigate back home.

Intriguingly, how lobsters, or any animal for that matter, perceive Earth's field is still unknown. Over the years, many researchers have attempted to localize the elusive magnetoreceptor, but no study to date has unambiguously identified a receptor. Much of this work has, however, provided insights into the potential mechanisms that allow animals to obtain information from the geomagnetic field. Two different mechanisms have been suggested: (1) the radical pairs hypothesis; and (2) the magnetite hypothesis. The radical pairs hypothesis suggests that animals might detect Earth's field through a complex series of light-induced chemical reactions (Ritz et al., 2000; Maeda et al., 2008; Liedvogel and Mouritsen, 2010). On the other hand, the magnetite hypothesis proposes that the geomagnetic field might be detected using microscopic crystals of the ferrimagnetic mineral magnetite (Fe_3O_4). In principle, because such crystals tend to align their dipole moments with Earth's field lines, information about the field might be transduced through the torque exerted on these crystals and activation of mechanically-activated receptors or ion channels (Kirschvink and Gould, 1981; Johnsen and Lohmann, 2005; Winklhofer and Kirschvink, 2010).

Of these two mechanisms, the magnetite hypothesis has accumulated the most widespread evidence phylogenetically. Magnetic particles have been found in the tissues of a number of animals, many of which are known to use the geomagnetic field as an orientation cue (Lohmann, 1984; Mann et al., 1988; Walker et al., 1997; Deibel et al., 2000). In attempts

to determine if such particles are associated with magnetoreception, pulse magnetization (a protocol in which animals are subjected to a brief, strong magnetic pulse) has been used extensively (Kirschvink et al., 2001; Shaw et al., 2015). This stimulus is capable of realigning the dipole moments of magnetite nanocrystals in magnetotactic bacteria (Kalmijn and Blakemore, 1978), and is thought to elicit similar effects in animals (Kirschvink, 1983; Kirschvink et al., 1985). Alternatively, a pulse might lead to disruption or damage of magnetite-based magnetoreceptors (Davila et al., 2005). In each case, receptor function should be altered, leading to erroneous perception of the geomagnetic field.

After animals are subjected to the magnetic pulse, researchers typically analyze subsequent orientation behavior for comparison with control animals (i.e., animals exposed to a sham pulse); if a difference is observed, this is often considered evidence for the magnetite hypothesis, as a magnetic pulse is not thought to have a lasting effect on a cryptochrome-based mechanism (Wiltschko et al., 2002). Numerous studies have used this technique; for example, effects have been reported in birds (Beason et al., 1995; Wiltschko et al., 1998, 2002; Holland, 2010; Holland and Helm, 2013), sea turtles (Irwin and Lohmann, 2005), mole rats (Marhold et al., 1997), and bats (Holland et al., 2008). These effects vary from shifts in migratory headings to induced random orientation in otherwise well-oriented animals. Nevertheless, although magnetic material has been found in many of the species shown to be affected by pulse magnetization, nothing is known about the physiological effects of the magnetic pulse treatment, or precisely how a pulse results in altered magnetic orientation behavior.

My dissertation focuses on magnetoreception in the Caribbean spiny lobster at multiple levels of organization, to gain insight into the mechanisms that underlie this elusive

sense. To this end, I took an interdisciplinary approach by examining both the behavioral and transcriptomic responses of spiny lobsters to high-intensity magnetic stimuli. Specifically, I: (1) examined how lobsters spontaneously respond to a novel magnetic anomaly; (2) investigated the effect of a strong magnetic pulse on lobster behavior; (3) constructed a *de novo* transcriptome assembly for the lobster central nervous system; and (4) elucidated how a magnetic pulse affects gene expression throughout the lobster central nervous system.

In the second chapter of my dissertation, "Size-dependent avoidance of a strong magnetic anomaly in Caribbean spiny lobsters," I investigate the spontaneous behavioral response of spiny lobsters to a strong magnetic anomaly. In this work, I demonstrate that lobsters avoid a strong magnetic anomaly and that lobster size is a significant predictor of avoidance behavior. These findings provide additional evidence for magnetoreception in spiny lobsters, raise the possibility of an ontogenetic shift in how lobsters respond to magnetic fields, and suggest that magnetic anomalies might influence lobster movement in the natural environment.

In my third chapter, "Effect of magnetic pulses on Caribbean spiny lobsters: implications for magnetoreception," I test the 'magnetite hypothesis' of magnetoreception by subjecting lobsters to a brief, strong magnetic pulse and examining their subsequent orientation behavior. Here, I demonstrate that a magnetic pulse alters subsequent orientation behavior, consistent with the hypothesis that magnetoreception in spiny lobsters is based at least partly on magnetite-based magnetoreceptors.

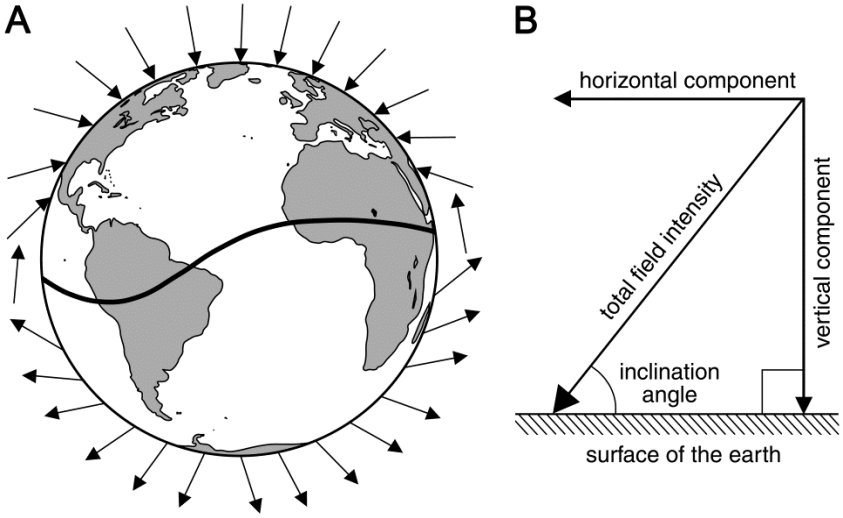
In my fourth chapter, "*De novo* assembly and annotation of a Caribbean spiny lobster central nervous system transcriptome," I use RNA-seq to create a transcriptome for analyzing gene expression in response to a magnetic pulse throughout the lobster central nervous

system. This work provides a novel resource for investigating the molecular mechanisms underlying magnetoreception in lobsters and is a key component of the work conducted in Chapter V.

In my fifth chapter, “The effects of a magnetic pulse on the spiny lobster central nervous system: transcriptomic insights for magnetoreception mechanisms,” I identify genes that show altered expression in response to a magnetic pulse in three different central nervous system tissues: the brain, subesophageal ganglion, and thoracic ganglia. In this work, I present evidence consistent with the hypothesis that a magnetic pulse affects iron-based receptors in the lobster central nervous system and uncover additional effects that suggest implications for interpreting the effects of a magnetic pulse on animal behavior.

Figures

Figure 1.1: Schematics of Earth's magnetic field (from Lohmann et al., 2007). (A) Diagram showing how the geomagnetic field lines vary with latitude. The inclination angle, or the angle at which the field lines intersect Earth's surface, is 0^0 at the magnetic equator (denoted by the curved line) and reaches $\pm 90^0$ at the magnetic poles. (B) The components of Earth's magnetic field. The total field intensity vector can be broken down into two vector components, horizontal and vertical field intensity.



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CHAPTER II

SIZE-DEPENDENT AVOIDANCE OF A STRONG MAGNETIC ANOMALY IN CARIBBEAN SPINY LOBSTERS[1]

Introduction

Animals rely on numerous sources of information for guidance while migrating, homing and moving around their habitats. Among these, the Earth's magnetic field is a particularly pervasive environmental feature, one that exists virtually everywhere on the planet. It is thus not surprising that diverse organisms, ranging from bacteria to vertebrate animals, have evolved ways to exploit the geomagnetic field to guide their movements (Wiltschko and Wiltschko, 1995; Johnsen and Lohmann, 2005).

Most studies on magnetoreception have focused on animals that derive directional or compass information from the Earth's field. A magnetic compass sense enables animals to maintain a consistent bearing, such as north or south. In addition, some animals can derive positional or 'map' information by detecting magnetic parameters such as intensity and inclination angle (the angle at which field lines intersect the Earth's surface), both of which vary predictably across the globe (Lohmann and Lohmann, 1994, 1996; Phillips et al., 2002; Lohmann et al., 2007).

For long-distance marine migrants such as sea turtles, salmon, and eels, the variation in Earth's magnetic field across the surface of the planet is sufficiently predictable that

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different oceanic regions and coastal locations can be identified by animals on the basis of distinctive magnetic signatures (Lohmann et al., 2001, 2012; Putman et al., 2013; Brothers and Lohmann, 2015; Naisbett-Jones et al., 2017). Nevertheless, local irregularities in the global pattern of geomagnetic variation exist. For example, in some locations, iron-containing rocks in the Earth's crust result in steep intensity gradients relative to the overarching regional magnetic field (Parkinson, 1983; Skiles, 1985; Lanza and Meloni, 2006). These gradients can be significantly stronger than the geomagnetic field; moreover, the direction of these local field gradients often differs from the overall pattern of the Earth's main dipole field (Lohmann et al., 2007).

Relatively little is known about how such magnetic anomalies affect animals. One possibility is that anomalies interfere with the normal functioning of magnetic compasses or maps and thus lead to disruptions in orientation and navigation. Consistent with this hypothesis, homing pigeons and migratory birds released at magnetic anomalies show signs of impaired orientation under some conditions (Walcott, 1978, 1992; Wiltschko et al., 2009, 2010; Schiffner et al., 2011). Alternatively or additionally, such anomalies might be exploited by animals as landmarks (Walker et al., 2002) or otherwise incorporated into navigational strategies. Several methods of navigation that rely at least partly on detecting naturally occurring magnetic intensity anomalies have been proposed for hammerhead sharks (Klimley, 1993), pigeons (Walker, 1998; Dennis et al., 2007) and whales (Klinowska, 1985; Kirschvink et al., 1986).

The Caribbean spiny lobster, *Panulirus argus* (Latreille 1804), is a benthic marine invertebrate that undertakes mass migrations (Kanciruk and Herrnkind, 1978; Herrnkind, 1980), is capable of homing (Creaser and Travis, 1950), and is known to exploit the Earth's

magnetic field for navigation (Lohmann et al., 1995; Boles and Lohmann, 2003; Ernst and Lohmann, 2016). Although numerous invertebrates extract directional information from the geomagnetic field, the spiny lobster is the only invertebrate known to also possess a magnetic map sense (Lohmann and Ernst, 2014). Because of their mobile lifestyle and regular migrations, spiny lobsters are likely to encounter magnetic anomalies in their environment, some resulting from natural geological formations and others from anthropogenic sources (e.g. submerged iron boat wreckage, oil platforms, and underwater cables).

As a first step toward determining whether lobster behavior is influenced by magnetic anomalies, we conducted a simple laboratory experiment in which lobsters were allowed to choose between sheltering in artificial dens that were either: (1) below sealed capsules containing neodymium magnets or (2) below identical capsules containing non-magnetic weights. Results indicated that lobsters spontaneously avoided strong magnetic anomalies and that avoidance behavior varied with size, inasmuch as lobsters that selected dens without magnets were significantly larger than those that occupied the magnet dens.

Materials and Methods

Animal collection and holding tanks

All experiments were conducted in Layton, FL, USA, at the Keys Marine Laboratory (24.83°N, 80.81°W) in July and August 2014. Lobsters with carapace lengths ranging from 42 to 88 mm were captured in Florida Bay within 350 m of the laboratory by swimmers using hand-held nets. Each animal was visually inspected for symptoms of *Panulirus argus* Virus 1 (PaV1), a viral infection that is prevalent in the Florida Keys and is known to affect lobster behavior. Animals that exhibited obvious signs of infection were not used in experiments.

After capture, lobsters were transported to the laboratory, where they were housed in rectangular, outdoor fiberglass holding tanks (122×67×39 cm) filled with flow-through seawater from Florida Bay. The ambient magnetic field within both holding tanks was measured with a triaxial fluxgate magnetometer (model 520A, Applied Physics Systems, Sunnyvale, CA, USA). The field had an inclination angle of 55.5° and an intensity of 43.7 μT. Each tank was shaded from the sun and contained a cement block that the lobsters used as a refuge. All lobsters were tested within 48 h of capture. The collection of lobsters was authorized by the Florida Fish and Wildlife Conservation Commission (permit SAL-11-1333D-SR).

Experimental arena

Lobsters were tested within a circular fiberglass tank (164 cm in diameter) filled with seawater to a depth of 23 cm. Measurements with the fluxgate magnetometer indicated that the ambient magnetic field in the arena had an inclination angle of 55.5° and an intensity of 43.7 μT, the same as in the holding tanks. Within the arena, concrete blocks (19×19×39.5 cm) were positioned to restrict lobsters to a rectangular channel (39.5×109.25 cm) oriented along the east–west axis within the center of the tank (Fig. 2.1). An additional concrete block was positioned at each end of the channel and against the wall of the tank, with the block openings (12×12 cm) oriented toward the center of the tank. This provided two artificial 'dens' at each end of the channel. A PVC capsule (2.54 cm diameter×7 cm length) containing either a cylindrical neodymium magnet (1.27 cm diameter×2.54 cm length; grade=N50; surface field=703.1 mT; Applied Magnets, Plano, TX, USA) or a nonmagnetic weight of similar size (control) was then centered on top of each concrete block so that the capsules

were flush with the edge of the block faces (Fig. 2.1). The capsules were sealed to prevent olfactory cues from the magnet or weight from entering the water.

Preference test

Lobsters were chosen at random and carefully placed in the center of the arena facing toward north and away from the person releasing the lobster. Thus, each lobster began its trial aligned perpendicular to the long axis of the arena and den openings. Lobsters were left alone in the arena for 15 min, after which time an observer returned to note the den that the lobster occupied.

After each trial, the water in the arena was thoroughly mixed so that residual odorants from lobsters tested previously were evenly dispersed throughout the tank. The water in the tank was completely changed at least once per day. In addition, the locations of the magnet and weight capsules were alternated between trials, and the north and south pole of the magnet were randomly oriented toward or away from the center of the channel (see Fig. 2.2 for magnet field intensity versus distance).

Each lobster was tested a single time. Prior to release, individuals were measured and sexed, and a semicircular notch was removed from one of the uropods. The notch permitted identification of lobsters that had been tested previously so that none were recaptured and tested multiple times.

All statistical analyses were conducted using R (Version 3.3.3, R Foundation for Statistical Computing, Vienna, Austria). Only lobsters that were within a den at the end of the 15-min trial (49 of 51) were included in the analysis. To investigate the relationship between size and den preference, I built a logistic regression model with carapace length as a predictor of den choice.

Results

Of the 49 lobsters that occupied a den by the end of the 15-min trial period, 33 (67%) occupied the control den and 16 (33%) occupied the magnet den (Fig. 2.3). Thus, lobsters showed a significant preference for the control den (exact binomial test, P=0.021). No relationship existed between sex and den preference (χ^2 test, χ^2=0.085, d.f.=1, P=0.77). In addition, male and female carapace length was not significantly different (Mann–Whitney U-test, U=227, P=0.2).

Lobsters that chose the magnet den were significantly smaller than those that chose the control den (Mann–Whitney U-test, U=142.5, P=0.0095; Fig. 2.4). Smaller lobsters had no apparent preference for either type of den, but as carapace length increased, the proportion of lobsters that chose the control den increased (Fig. 2.5). Moreover, a logistic regression model showed that carapace length is a significant predictor of den choice (P=0.019; Fig. 2.6, Table 2.1).

Discussion

In a two-choice preference test, significantly more lobsters selected the control den than selected the den associated with the neodymium magnet (Fig. 2.3). The overall aversion to dens with magnets was driven primarily by the behavior of the larger lobsters, which showed a strong preference for control dens (Figs 2.4, 2.5, Table 2.1); by contrast, smaller lobsters as a group lacked a preference for den type. Although previous studies have revealed that spiny lobsters detect and respond to Earth-strength magnetic fields (Lohmann et al., 1995; Boles and Lohmann, 2003), these findings are the first to demonstrate that they also detect and respond to a localized magnetic anomaly produced by a magnet.

What field component(s) do lobsters detect?

The results demonstrate that lobsters detect and avoid the magnetic fields produced by magnets, but they do not reveal the precise element(s) of the magnetic field that lobsters detect. The magnetic field that naturally exists at any location on Earth can be described in terms of total field intensity and inclination angle (Lohmann et al., 1999). The same is true for the field produced by a magnet, with the caveat that the magnet's field, unlike the natural geomagnetic field, has a strong gradient (i.e., both field strength and inclination vary greatly over a short distance). Thus, in principle, lobsters might detect one or more of the following: (1) the total intensity of the field produced by the magnet; (2) the inclination angle; (3) the intensity gradient; (4) the inclination gradient; or (5) the range of field directions within the horizontal plane when close to the magnet. Additional changes in field components such as horizontal and vertical intensity also occur close to a magnet, but whether any animal can resolve the total field into vector components is not known.

Why avoid dens with magnets?

The reason why lobsters avoided dens with magnets is not known, but several explanations are plausible. Given that lobsters are known to possess 'magnetic maps' and can thus assess their geographic location relative to 'home' (Boles and Lohmann, 2003), one possibility is that lobsters preferred dens with ambient magnetic fields similar to those of their home area. Because the test arena was located within 350 m of the capture site, the control den had a magnetic field nearly identical to that of the capture location. As lobsters approached the magnet den, they might have interpreted the anomalous field to mean that they were far from home, resulting in exploration that led eventually to the discovery of the control den and the more familiar magnetic field.

Another possibility is that lobsters avoided dens with magnets because the anomalous field represented an unnatural and unfamiliar magnetic environment. For example, some of the inclination and intensity values near the magnet were presumably outside the range that a lobster in Florida would normally encounter; moreover, the magnet created steep gradients in magnetic parameters, so that as a lobster approached a den with a magnet, the intensity, inclination, and direction of the field all changed rapidly. Lobsters might thus have found the magnet's field to be confusing or disturbing. In addition, the magnetic gradient produced by the magnet might conceivably have generated unusual or uncomfortable sensations through effects on the lobster magnetoreceptor system. Magnetic material has been detected in spiny lobsters (Lohmann, 1984), and experiments with magnetic pulses suggest that this material might provide the physical basis for magnetoreception (Ernst and Lohmann, 2016). Because magnetic particles experience a force in a magnetic gradient (Oldenburg et al., 2005), an interesting speculation is that lobsters approaching magnets experienced unusual or uncomfortable sensations resulting from forces exerted on magnetite-based magnetoreceptors, and therefore moved away.

Size-dependent magnetic field avoidance

Interestingly, the behavioral response of lobsters to a strong magnetic anomaly was size dependent: larger lobsters avoided dens with magnets whereas smaller lobsters appeared indifferent to magnets when choosing a den. These findings are consistent with the hypothesis that lobsters undergo a size-dependent shift in their behavioral response to magnetic fields, although additional studies are needed to confirm or refute this possibility.

The reason for the size-dependent aversion to magnets remains unknown, but an interesting possibility is that larger lobsters might be more sensitive to magnetic stimuli than

smaller lobsters. Consistent with this idea, the magnetic remanence (a measure of the quantity of magnetic material present) of the lobster cephalothorax and abdomen increased with carapace length (Lohmann, 1984), as might be expected if larger, more mature lobsters have a more developed magnetite-based magnetoreceptor system. An alternative explanation for the present results, however, is that smaller lobsters are under greater risk of predation than larger lobsters (Andree, 1981; Eggleston and Lipcius, 1992; Smith and Herrnkind, 1992) and might therefore be more strongly motivated to take cover, even in suboptimal dens. By contrast, larger lobsters less vulnerable to predation might be more willing to explore further until encountering a den with more favorable magnetic conditions. Regardless, further studies will be needed to investigate the cause of the observed size effect.

Natural anomalies versus magnet anomalies

Although our results provide the first evidence that spiny lobsters detect and respond to a strong magnetic anomaly, the anomalies used in this study were greater in intensity, and had stronger gradients, than naturally occurring anomalies caused by geological formations. For this reason, and because strong fields might hypothetically affect physiological processes that are unaffected by weaker fields, caution is needed in extrapolating the results to the natural behavior of lobsters. Nevertheless, if lobsters do indeed generally avoid magnetic anomalies, then an interesting speculation is that the animals might avoid geographic areas where the ambient field varies irregularly, perhaps because such conditions make it difficult for the animals to guide themselves using their magnetic compasses (Lohmann et al., 1995) and magnetic maps (Boles and Lohmann, 2003). Further studies will be needed to investigate how lobsters respond to more natural magnetic anomalies and whether they attempt to circumvent them when possible.

Responses of animals to magnets

Most studies on animal magnetoreception have involved magnetic fields that closely resemble the natural field of the Earth. Indeed, an increased emphasis in recent years has been on developing coil systems that generate highly uniform fields and minimize anomalies (Kirschvink, 1992), a trend fueled by the belief that animals will not spontaneously respond to magnetic fields unlike those that exist in nature. However, a growing body of literature suggests that at least some animals can detect the fields of magnets and respond to them either spontaneously (Brown et al., 1960a,b; Kremers et al., 2014; O'Connell et al., 2015; Vidal-Gadea et al., 2015) or after being conditioned to do so (Thalau et al., 2007; Denzau et al., 2011; Freire et al., 2012). If spontaneous responses similar to those we have observed in lobsters turn out to be widespread phylogenetically, then exposing animals to the fields of magnets might provide a new and useful assay of magnetic sensitivity.

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Tables

Table 2.1: Logistic regression model statistics, with carapace length as a predictor of den choice.

Variable	**Coefficient**	**s.e.m.**	**Odds ratio**	**2.5% CI**	**97.5% CI**	***z***	***P*-value**
Intercept	-5.34	2.53	0.005	1.93×10^{-5}	0.45	-2.11	0.035*
Carapace length	0.11	0.05	1.11	1.03	1.23	2.36	0.019*

CI, confidence interval; * $P<0.05$.

Figures

Figure 2.1: Schematic of the experimental arena. Each sealed PVC capsule contained either a neodymium magnet or a non-magnetic weight of similar size.

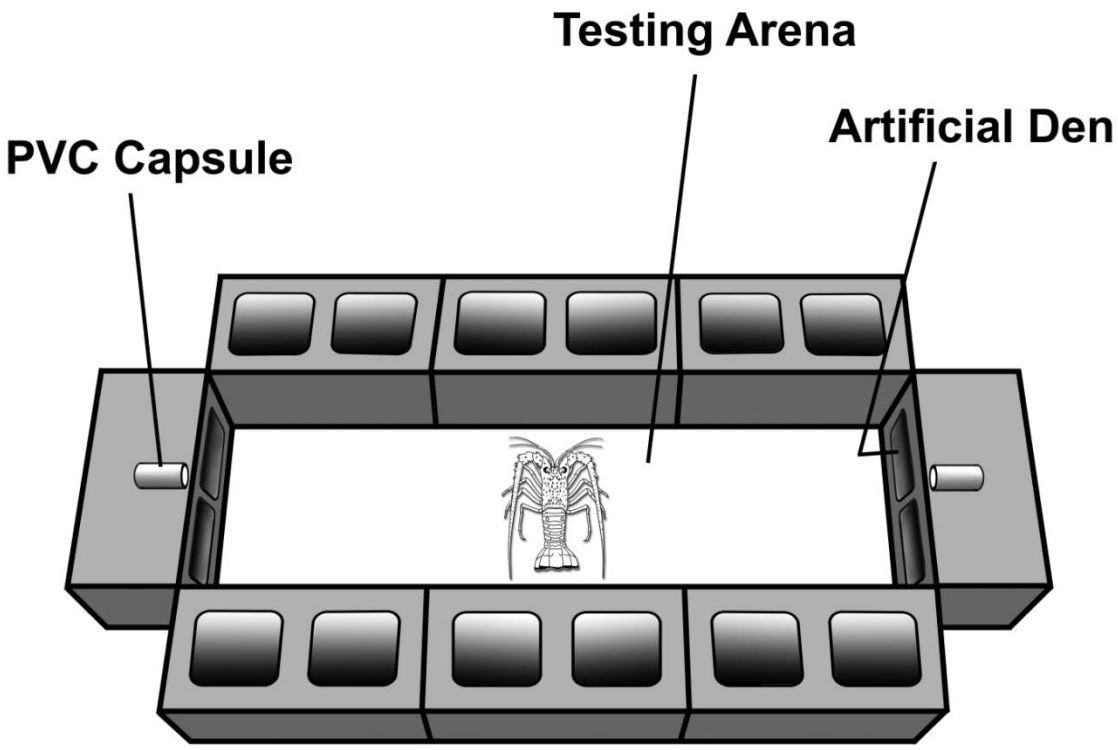


Figure 2.2: Approximate magnetic field strength experienced by lobsters as a function of distance from the magnet's surface. All values were calculated for positions on a horizontal plane that approximated the height of a walking lobster; the plane was 10.5 cm above the floor of the arena and 10.5 cm below the midway point of the magnet positioned on top of the concrete block (see diagram at bottom of figure; MAG, den with magnet; CON, den with nonmagnetic weight; white rectangles on dens indicate PVC capsules). 0 on the x-axis indicates a position 10.5 cm directly below the magnet surface. The gray vertical dashed line at 55 indicates the midpoint of the arena (i.e. the lobster release location). Measurements taken with a DC magnetometer (AlphaLab, Inc., West Salt Lake City, UT, USA) at various distances from the magnet showed good agreement with the calculated values and were used to spot-check the general accuracy of the graph. Both calculations and measurements indicated that, at the level where the lobsters walked, the field produced by the magnet was essentially zero beyond the midpoint of the arena.

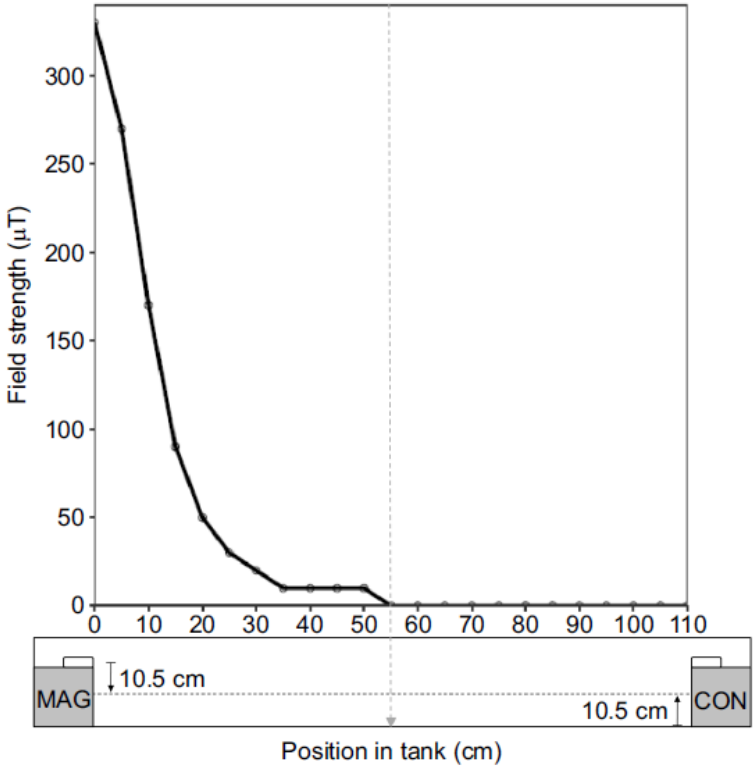


Figure 2.3: Total numbers of lobsters that occupied each den type. Significantly more lobsters took refuge in control dens (*n*=33) than in dens below a magnet (*n*=16) (*P*=0.021, exact binomial test). Control, non-magnetic weight; magnet, neodymium magnet.

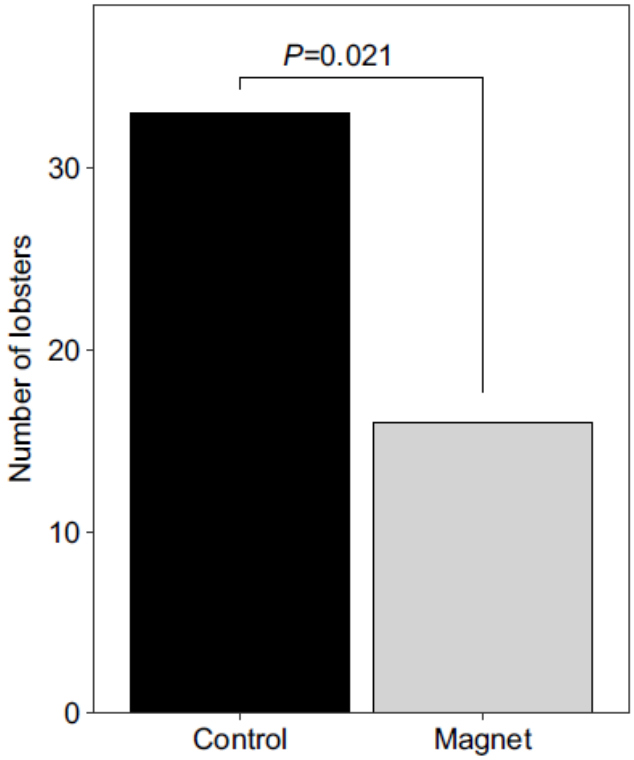


Figure 2.4: Mean carapace length of lobsters that occupied each den type. Lobsters that selected control dens (*n*=33) were significantly larger on average than those (*n*=16) that selected magnet dens (*P*=0.0095, Mann–Whitney *U*-test). Error bars indicate s.e.m.

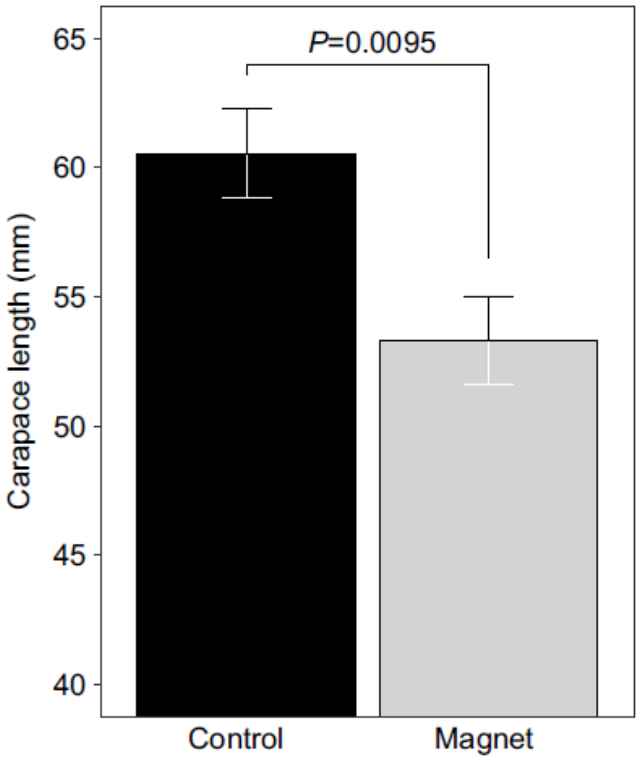


Figure 2.5: Den choice across 10 mm carapace length bins. (A) Proportion of lobsters that occupied each den type. (B) Total number of lobsters that chose each den type (*n*=49).

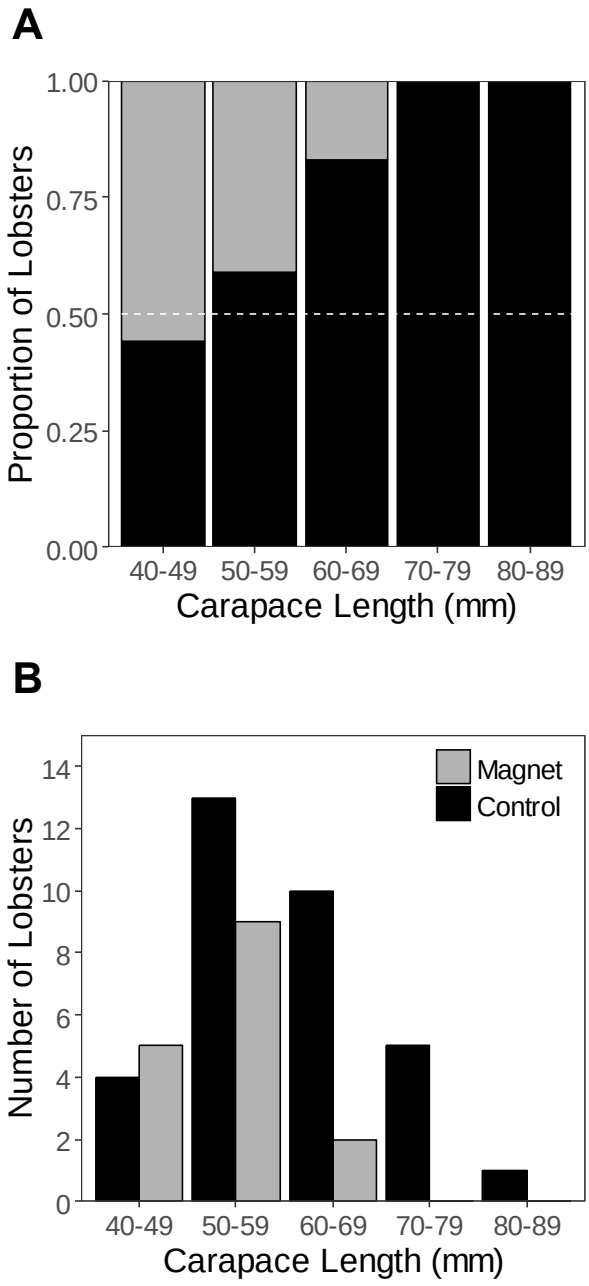
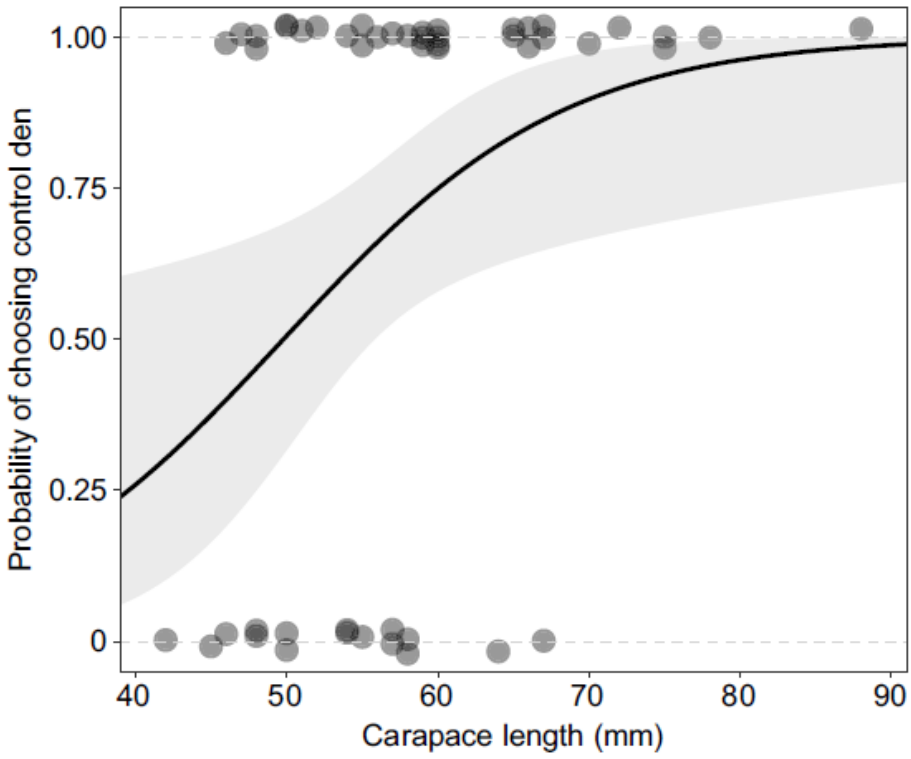


Figure 2.6: Logistic regression curve showing the relationship between carapace length and den choice. Carapace length is a significant predictor of den choice (P=0.019). Each circle represents an individual lobster, with circles along the 1.00 line representing lobsters that chose the control den (n=33) and points along the 0 line denoting lobsters that chose the den with the magnet (n=16). Circles are offset and transparent for clarity. The shaded area represents the 95% confidence interval.



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CHAPTER III

EFFECT OF MAGNETIC PULSES ON CARIBBEAN SPINY LOBSTERS: IMPLICATIONS FOR MAGNETORECEPTION[2]

Introduction

Earth's magnetic field plays an important role in guiding the movements of diverse animals over a wide range of distances (Wiltschko and Wiltschko, 2005; Johnsen and Lohmann, 2005). The geomagnetic field is among the most pervasive and reliable of orientation cues, accessible day and night at nearly every location on the planet. Animals can derive two distinct types of information from the geomagnetic field. Many species use the field as a source of directional or 'compass' information, which enables them to maintain consistent headings (e.g., toward north or south) (Lohmann, 2010). In addition, Earth's magnetic field varies predictably across the surface of the globe, providing a source of positional or 'map' information that some animals use to change direction at appropriate locations along a migratory route or to navigate toward particular geographic areas (Lohmann et al., 2001, 2004, 2007, 2012; Phillips et al., 2002; Putman et al., 2014).

Although many species evidently use the geomagnetic field as a compass, map, or both, the transduction mechanisms that underlie magnetic field detection have not been clearly established in any animal. Several different hypotheses have been proposed to explain how animals might detect magnetic fields (Johnsen and Lohmann, 2008). Most recent

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research has focused on two possible biophysical mechanisms: (1) chemically mediated magnetoreception (Ritz et al., 2000; Maeda et al., 2008; Liedvogel and Mouritsen, 2010); and (2) magnetite-based magnetoreception (Kirschvink et al., 2001; Walker, 2008; Winklhofer and Kirschvink, 2010).

The magnetite hypothesis proposes that particles of the mineral magnetite (Fe_3O_4) provide the physical basis for the magnetic sense. Theoretical considerations suggest that single-domain magnetite crystals (crystals of a size that can sustain a permanent magnetic moment) are particularly well suited to function as magnetoreceptors (Kirschvink et al., 2001). Such particles might activate secondary receptors (e.g., stretch receptors or hair cells) as the particles twist into alignment with the geomagnetic field (Kirschvink and Gould, 1981; Johnsen and Lohmann, 2005; Winklhofer and Kirschvink, 2010). Magnetic particles have been detected in the tissues of a number of animals, many of which use the geomagnetic field as an orientation cue (e.g., Lohmann, 1984; Mann et al., 1988; Walker et al., 1997; Shaw et al., 2015).

One technique that has been used to investigate magnetite-based magnetoreception involves subjecting organisms to brief, strong magnetic pulses (Kirschvink et al., 2001; Shaw et al., 2015), a treatment that should have no lasting effect on chemically mediated magnetoreception (Wiltschko et al., 2002). In principle, a strong magnetic pulse applied in the right direction can realign the magnetic dipole moment of a single-domain magnetite crystal (Kirschvink, 1983; Kirschvink et al., 1985). As a consequence, the pulse treatment might cause incorrect magnetic information to be transduced to the nervous system, resulting in changes in orientation behavior. Magnetic pulses have been shown to alter the orientation of several vertebrate animals, including sea turtles (Irwin and Lohmann, 2005), migratory

birds (Beason et al., 1995; Wiltschko et al., 1998, 2002; Holland, 2010; Holland and Helm, 2013) and mammals (Marhold et al., 1997a; Holland et al., 2008). In some cases, the treatment has disrupted existing directional preferences, resulting in random orientation; in others, it has elicited shifts in preexisting directional preferences.

To my knowledge, all of the animals used in magnetic pulse experiments so far have been vertebrates; whether invertebrate animals are also affected by magnetic pulses has not been investigated. In the context of magnetoreception, a particularly interesting invertebrate is the Caribbean spiny lobster, *Panulirus argus* (Latreille 1804), the only invertebrate species known to have both a magnetic compass (Lohmann et al., 1995) and a magnetic map (Boles and Lohmann, 2003; Lohmann and Ernst, 2014). Spiny lobsters undergo an annual mass migration and are capable of homing after nocturnal foraging or experimental displacements (Creaser and Travis, 1950; Herrnkind and McLean, 1971; Herrnkind and Redig, 1975; Herrnkind et al., 1975). In addition, concentrations of permanently magnetic material thought to be magnetite have been detected in the Caribbean spiny lobster (Lohmann, 1984).

As a first step toward determining whether magnetic particles are associated with magnetoreception in the spiny lobster, I studied the orientation behavior of lobsters subjected to strong magnetic pulses. Results indicated that a magnetic pulse altered subsequent orientation, a finding consistent with the hypothesis that magnetoreception in lobsters is based at least partly on magnetite-based magnetoreceptors.

Materials and Methods

Animals

All experiments were conducted in Layton, Florida, USA, at the Keys Marine Laboratory (24.83°N, 80.81°W) in July 2013. Juvenile lobsters ranging from 55 to 86 mm in

carapace length were captured in Florida Bay in the immediate vicinity of the laboratory by swimmers using hand-held nets. Each animal was visually inspected for signs of ill health. Healthy lobsters were placed into plastic buckets (18.9 liters) filled with seawater and transported to the laboratory for experiments. Those few animals that showed symptoms of PaV1 (*Panulirus argus* Virus 1, a virus that infects spiny lobsters) or other disease were not used. The collection of lobsters was authorized by the Florida Fish and Wildlife Conservation Commission (permit SAL-11-1333C-SR).

Magnetic pulse protocol

Lobsters were collected daily between 18:00 and 20:00 h and randomly assigned to one of three groups. Within 1 h of capture, lobsters in two of the groups were exposed to strong magnetic pulses (see below), while those in the third group (controls) were handled in the same way as the others, but not exposed to a magnetic pulse.

Magnetic pulses were generated by a magnetizer (model 7515-G) constructed by Magnetic Instrumentation (Indianapolis, IN, USA). The magnetizer consisted of a bank of capacitors (425 V max) that discharged to a solenoid (32 cm diameter×20 cm length). Magnetic pulses produced by the magnetizer had an intensity of 85 mT and a duration of 5 ms. Both values are within the range used in similar studies with other animals (Irwin and Lohmann, 2005; Holland et al., 2008; Holland, 2010; Holland et al., 2013; Holland and Helm, 2013).

Prior to placing the lobsters in the solenoid of the magnetizer, eye caps molded from polyvinylsiloxane impression material (Kerr Manufacturing Co., Orange, CA, USA) were placed over the lobsters' eyestalks to obscure their vision. Each lobster was then fastened to a small wooden board (approximately 5×75×2.5 cm, width×length×depth) with plastic cable

ties. The board and lobster were then placed on non-magnetic supports and positioned so that the lobster was centered within the solenoid of the magnetizer and aligned along the magnetic north–south axis.

Because the effect of a magnetic pulse on magnetite crystals depends in part on how crystals are aligned relative to the pulse direction (Wiltschko et al., 2002; see Discussion), lobsters were treated under two sets of conditions. One group of lobsters was subjected to a magnetic pulse directed from posterior to anterior, with the pulse delivered parallel to the geomagnetic horizontal component (i.e., toward magnetic north; Fig. 3.1A). A second group was also subjected to a magnetic pulse directed from posterior to anterior, but with the pulse delivered antiparallel to the geomagnetic horizontal component (i.e., toward magnetic south; Fig. 3.1B). An additional group of control lobsters was eye-capped, fastened to the wooden board, and placed inside the solenoid, but not subjected to a magnetic pulse.

After lobsters were removed from the solenoid and detached from the wooden board, the eye caps were removed and lobsters were then housed outdoors in two rectangular fiberglass holding tanks (67×122×39 cm) placed side by side and filled with flow-through seawater from Florida Bay. Each tank was shaded from the sun and contained a concrete block that the lobsters could use for cover. The two tanks appeared to be identical. Nevertheless, to ensure that the tank in which lobsters were housed did not influence the outcome, treatment groups were assigned to different holding tanks on different days of the experiment. The water temperature in both holding tanks was equivalent to that of Florida Bay.

All lobsters remained in the tanks overnight (for at least 10 h). The next morning, each lobster was tested a single time in the orientation arena (see below) and then released.

Orientation trials

Lobsters were housed and tested in the local magnetic field. The field was measured with a triaxial magnetometer (model 520A, Applied Physics Systems, Sunnyvale, CA, USA) and determined to have an intensity of 43.8 μT and an inclination of 53.7°.

All orientation trials were conducted between 07:00 and 14:00 h at a location approximately 200 m southeast of the capture site. Before testing, each lobster was eye-capped to eliminate the use of visual cues. A plastic cable tie was secured around the posterior cephalothorax between the fourth and fifth pairs of pereopods. A small plastic ring (1 cm diameter) threaded onto the cable tie was positioned along the lobster's dorsal midline as an attachment point for a tether.

Lobsters were tethered with monofilament line within a circular, water-filled fiberglass arena (164 cm diameter; 29 cm water depth). One end of the tether was attached to a non-magnetic brass fishing swivel, which in turn was connected to the plastic ring on the midline of the lobster. The other end was attached to an electronic tracking system positioned on a support beam that extended across the center of the arena (Fig. 3.2). The tracking system consisted of a rotatable tracker arm, capable of pointing toward any direction in the horizontal plane, affixed to a digital encoder that transmitted the angle of orientation to a computer for data collection. The tether restrained lobsters to a circle with a radius of 25.5 cm.

Once tethered, lobsters were released randomly in one of the four cardinal directions and allowed to walk on a level, circular piece of acrylic positioned on the bottom of the tank. When the tether became taut, animals continued to walk at the same steady rate with their

legs slipping continuously on the acrylic surface (Lohmann et al., 1995). The trial was then initiated, and each lobster's heading was recorded every 30 s for a period of 30 min.

After testing and prior to release, a circular notch was taken out of each lobster's right uropod. This ensured that each lobster could be identified upon recapture and that no lobster was inadvertently tested a second time.

All orientation experiments were carried out during a 7-day period (2–8 July) in 2013. The experiment was conducted in two phases. During the first 5 days, control lobsters and lobsters subjected to the antiparallel pulse were tested alternately in the arena; in other words, the first lobster tested was a control, the second was from the antiparallel pulse group, the third was a control, and so on. During the last 2 days of the experiment, I tested an additional group of lobsters that had been subjected to a parallel pulse.

Statistical analysis

Using standard procedures for circular statistics (Batschelet, 1981), a mean angle for each lobster was calculated based on all measurements obtained during the 30-min trial. Rayleigh tests were used to determine whether each group of lobsters was significantly oriented. The distributions of the three groups were compared using the Mardia–Watson–Wheeler test; pairwise comparisons were made with the Watson test (Batschelet, 1981; Zar, 1999). In addition, to determine whether individual lobsters in some treatment groups held more consistent headings than lobsters in other groups, individual *r*-values (indicators of directional consistency) were compared across groups using a Kruskal–Wallis *H*-test (Siegel and Castellan, 1988).

Results

Lobsters exposed to a magnetic pulse directed parallel to the horizontal component of the geomagnetic field (Fig. 3.1A) were significantly oriented with a mean angle of 259° (Rayleigh test, n=15, r=0.45, Z=2.98, P=0.048; Fig. 3.3A). Lobsters exposed to a magnetic pulse directed antiparallel to the geomagnetic field (Fig. 3.1B) were significantly oriented with a mean angle of 47° (Rayleigh test, n=14, r=0.53, Z=3.98, P=0.016; Fig. 3.3B), a direction approximately opposite that of the first group. By contrast, control lobsters (lobsters not exposed to a magnetic pulse) had orientation that was statistically indistinguishable from random (Rayleigh test, n=13, r=0.26, Z=0.886, P=0.42; Fig. 3.3C).

Significant differences existed among the three distributions (Mardia–Watson–Wheeler test, W=15.036, P=0.005). Pairwise comparisons indicated that the distributions of the parallel and antiparallel pulsed groups were significantly different (Watson test, U^2=0.323, P<0.005). In addition, the antiparallel group and control group were significantly different (Watson test, U^2=0.211, P<0.05). The distributions of the parallel group and control group were not significantly different (Watson test, U^2=0.091, P>0.2). A comparison of the r-values of individual lobsters (calculated using all bearings recorded during the 30-min trial period) did not reveal any significant difference among the three groups (Kruskal– Wallis H-test, H=1.928, P=0.381), indicating similar levels of directional consistency regardless of treatment.

Discussion

The results indicate that a magnetic pulse affected the subsequent orientation behavior of spiny lobsters. Control lobsters placed into the solenoid of the magnetizer, but not subjected to a magnetic pulse, were not significantly oriented as a group (Fig. 3.3C). By

contrast, the two groups of lobsters that were exposed to a magnetic pulse each showed a significant directional preference, with the preferred direction apparently influenced by the alignment of the animal and magnetic pulse relative to Earth's magnetic field (Fig. 3.3A,B). The finding that a magnetic pulse affected orientation is consistent with the hypothesis that lobsters have magnetite-based magnetoreceptors (Kirschvink et al., 2001; Johnsen and Lohmann, 2005). Indeed, of the various mechanisms that have been proposed to underlie magnetoreception, only magnetite should hypothetically be affected by a strong magnetic pulse (Shaw et al., 2015).

All of the lobsters in this study were tested at a location within approximately 200 m of where they were captured. The lack of a directional preference in control lobsters is consistent with previous results, in which lobsters tethered in an underwater arena close to the capture site failed to orient consistently as a group, possibly because the animals were already in the immediate vicinity of their home dens (Lohmann et al., 1995).

Magnetic pulses similar to those used in the present study have been reported to alter the orientation behavior of several vertebrate animals, including sea turtles (Irwin and Lohmann, 2005), birds (Beason et al., 1995; Wiltschko et al., 1998, 2002; Holland, 2010; Holland and Helm, 2013) and mammals (Marhold et al., 1997a; Holland et al., 2008). The present study provides evidence that a magnetic pulse can also alter the orientation behavior of an invertebrate animal.

Effect on magnetic map or magnetic compass?

Spiny lobsters are able to derive both directional ('compass') information and positional ('map') information from Earth's magnetic field (Lohmann et al., 1995; Boles and

Lohmann, 2003). In principle, the magnetic pulse might have altered or impaired mechanisms underlying one or both of these abilities.

In migratory birds, a magnetic pulse has been hypothesized to affect a magnetite-based map sense. Birds that have completed at least one migration are thought to acquire a map through experience, whereas first-time migrants are thought to follow a consistent compass heading that does not require a map (Wiltschko and Wiltschko, 1995a,b, 2003). Consistent with this hypothesis, a magnetic pulse affected the orientation of Australian silvereyes (*Zosterops lateralis lateralis*) that had migrated at least once before, but had no effect on naïve birds migrating for the first time (Wiltschko et al., 1994, 1998; Munro et al., 1997).

In lobsters, one possibility is that the magnetic pulse altered magnetite-based receptors associated with a magnetic map sense (Lohmann et al., 2007), causing lobsters to perceive positional information incorrectly. If so, then an interesting speculation is that lobsters in the parallel pulse condition might have perceived erroneously that they had been displaced east of the capture site, whereas lobsters in the antiparallel pulse condition might have perceived themselves to be southwest of the capture site, resulting in attempts to home in opposite directions. Additional studies will be needed to confirm or refute this hypothesis.

Additionally or alternatively, it is possible that the magnetic pulse affected the magnetic compass. Interestingly, the lobster compass has different functional properties from those of several other animals including birds (Wiltschko and Wiltschko, 1972), sea turtles (Light et al., 1993; Goff et al., 1998) and monarch butterflies (Guerra et al., 2014). Unlike lobsters, these animals all have inclination or axial compasses that are apparently blind to field polarity (Wiltschko and Wiltschko, 1972) and have properties compatible with chemical

magnetoreception (Wiltschko and Wiltschko, 2010). By contrast, lobsters have a polarity compass with properties incompatible with chemical magnetoreception but consistent with magnetite (Lohmann et al., 1995; Johnsen and Lohmann, 2005; Lohmann and Ernst, 2014). It is noteworthy that mole rats and bats also have a polarity compass (Marhold et al., 1997b; Wang et al., 2007) and show altered orientation after a magnetic pulse (Marhold et al., 1997a; Holland et al., 2008), consistent with a magnetite-based compass in these animals.

Although it is also hypothetically possible that a magnetic pulse might affect orientation behavior via a general effect on lobster physiology, health or motivation, we consider this unlikely for several reasons. First, the finding that lobsters oriented in approximately opposite directions, depending on the direction of the magnetic pulse, is difficult to reconcile with a non-specific effect. Second, a significant recovery period (at least 10 h) elapsed between exposure to the magnetic pulse and orientation tests. Finally, no general effects of a magnetic pulse on physiology or behavior have been reported in similar experiments with other animals (Wiltschko et al., 1994, 1998, 2002; Beason et al., 1995, 1997; Wiltschko and Wiltschko, 1995b; Munro et al., 1997; Irwin and Lohmann, 2005; Holland et al., 2008).

Magnetoreceptor structure

Although evidence for magnetite-based magnetoreception has been accumulating, the exact structure of the putative receptors remains speculative. Hypothetically, a single-domain magnetite crystal able to rotate freely will continuously align itself with the direction of the ambient field (Johnsen and Lohmann, 2005). Little is known, however, about whether magnetite crystals are free to rotate or are instead restricted to a narrow range of movement. In some models of magnetoreceptors, magnetite crystals can align in any direction

(Kirschvink and Gould, 1981). In others, they are anchored in place and can move only over a limited range (Walker, 2008; Winklhofer and Kirschvink, 2010; Lohmann, 2016). The extent to which a magnetite particle can move has implications for how magnetite interacts with secondary receptors or ion channels and how the torque of a magnetite particle is ultimately converted into electrical signals during the transduction process for the magnetic sense.

In principle, a single-domain magnetite crystal subjected to a strong magnetic pulse directed parallel to the crystal's magnetic moment should remain functionally unchanged. By contrast, if a magnetic pulse is delivered antiparallel to the crystal's magnetic moment, the polarity of the magnetic moment will be reversed (Kirschvink, 1983; Kirschvink et al., 1985). Thus, the effect of a magnetic pulse depends on the alignment of the pulse relative to the dipole moment of a magnetite crystal.

In the present experiment, it is unclear whether magnetite crystals were able to rotate into alignment with the geomagnetic field prior to the pulse. This issue is of particular interest in the context of the parallel pulse group, which had a significant directional preference (Fig. 3.3A) whereas controls did not (Fig. 3.3C). One possibility is that some magnetite particles were unable to align with the geomagnetic field and were thus remagnetized in the opposite direction, resulting in altered orientation behavior as reported in some similar experiments with birds (Beason et al., 1995; Wiltschko et al., 2002). However, because the orientation of the parallel and control groups were not significantly different for the lobsters, caution is required in interpreting this part of the experiment and no firm conclusions can be drawn.

Regardless, the finding that magnetic pulses alter orientation responses in lobsters is consistent with magnetoreceptors based on single-domain magnetite crystals. Future work will be needed to definitively characterize the mechanisms that underlie magnetoreception in lobsters and other animals.

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Figures

Figure 3.1: Magnetic pulse treatment. All lobsters were placed tail-first into the solenoid of the magnetizer. (A) Parallel magnetic pulse condition: lobsters were treated with a magnetic pulse directed parallel to the horizontal component of the geomagnetic field (i.e., toward magnetic north) while facing north. (B) Antiparallel magnetic pulse condition: lobsters were treated with a magnetic pulse directed antiparallel to the horizontal component of the geomagnetic field (i.e., toward magnetic south) while facing south. The cylinder represents the solenoid while the arrow outside the solenoid indicates the direction of the magnetic pulse (N, north; S, south).

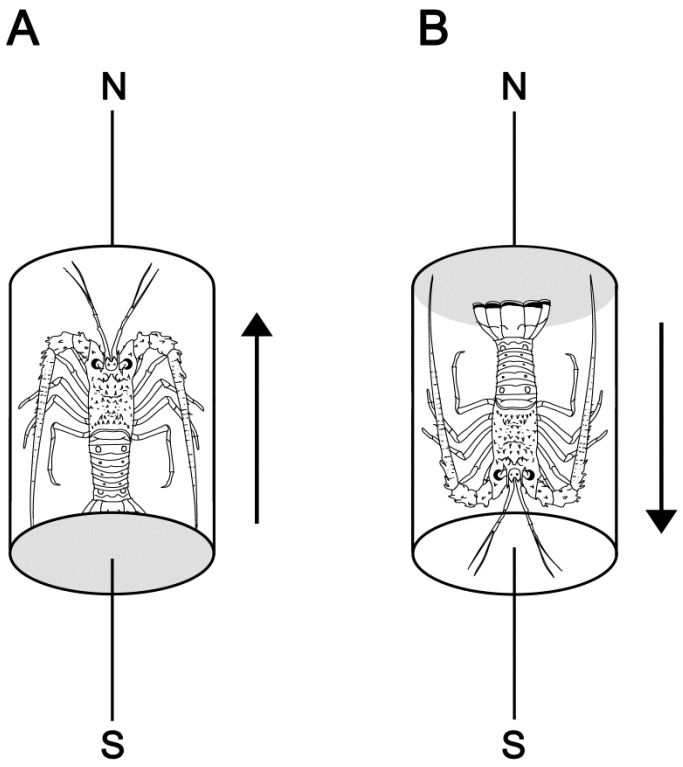


Figure 3.2: Orientation arena. Lobsters were tethered within a circular arena to an electronic tracking system consisting of a tracker arm, digital encoder (black box above the lobster) and computer that monitored the angle of orientation. See Materials and Methods for details.

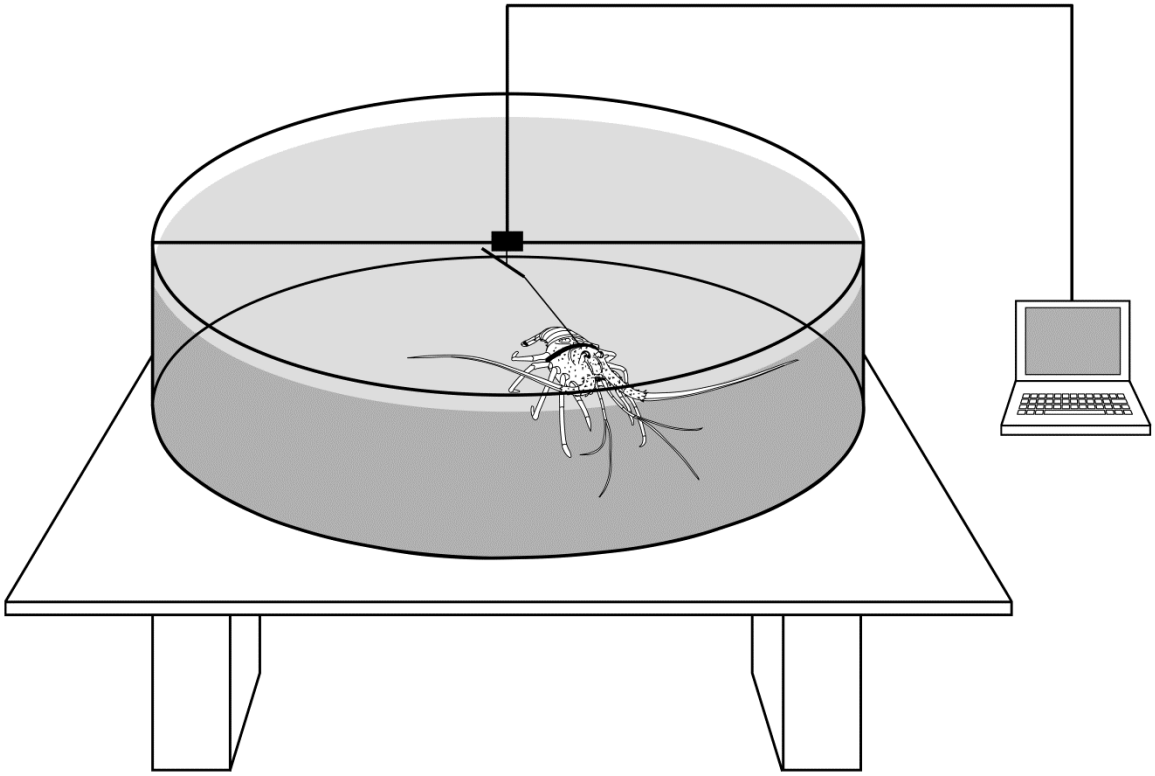
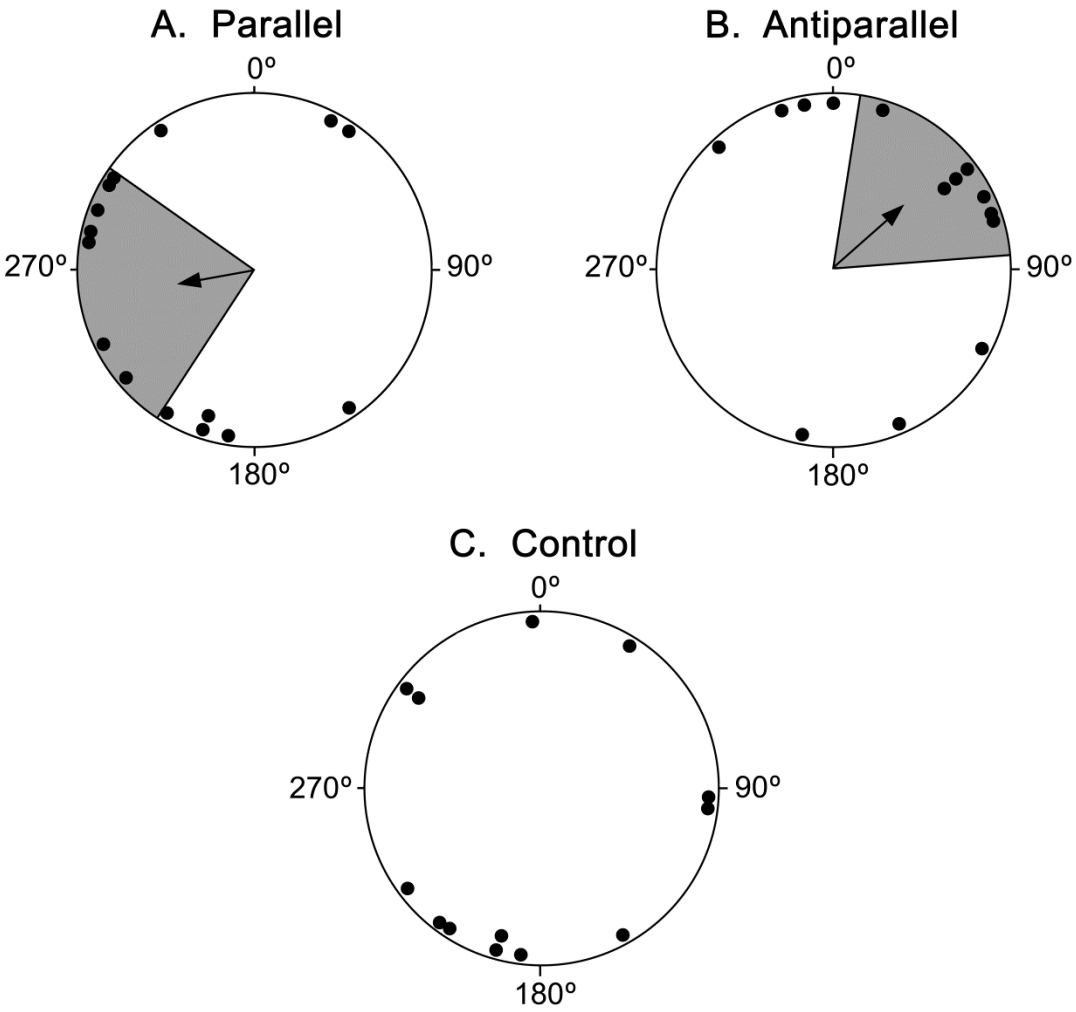


Figure 3.3: Lobster orientation trial results. (A) Lobsters treated with a pulse directed parallel to the geomagnetic field were significantly oriented with a mean angle of 259°. (B) Lobsters treated with a pulse directed antiparallel to the geomagnetic field were significantly oriented in approximately the opposite direction, with a mean angle of 47°. (C) Control lobsters were not oriented as a group. Each black circle represents the mean heading of an individual lobster. Arrows indicate the mean direction of the group. Shaded areas represent the 95% confidence interval for the mean.



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CHAPTER IV

DE NOVO ASSEMBLY AND ANNOTATION OF A CARIBBEAN SPINY LOBSTER CENTRAL NERVOUS SYSTEM TRANSCRIPTOME

Introduction

Crustaceans comprise an exceptionally large and diverse group of animals, one that has proven useful in investigating a variety of biological questions. Crustacean model systems have played pivotal roles in neuroscience research, notably in the discovery of electrical synapses and command neurons in crayfish (Furshpan and Potter, 1959; Wiersma and Ikeda, 1964), as well as advances in understanding central pattern generator circuits and neural modulation (Hooper and DiCaprio, 2004). In addition, crustaceans are important for the study of numerous sensory modalities, including chemoreception (Derby and Weissburg, 2014; Derby et al., 2016), vision (Glantz, 2014; Cronin and Feller, 2014), and magnetoreception (Lohmann and Ernst, 2014). To date, however, few crustacean genomes have been sequenced, which has impeded efforts to unravel the molecular basis of neural functions and sensory transduction mechanisms. Nevertheless, the recent development of next-generation sequencing (NGS) technologies, especially sequencing the transcribed elements of the genome (e.g., RNA-seq; Wang et al. 2009), has enabled the investigation of novel biological questions in non-model species.

The Caribbean spiny lobster, *Panulirus argus*, is a promising model system for investigating the molecular mechanisms underlying magnetoreception. This species is the only invertebrate known to derive both directional and positional information from the

geomagnetic field (Lohmann et al., 1995; Boles & Lohmann, 2003). In addition, these lobsters avoid strong magnetic anomalies (Ernst and Lohmann, 2018), and their orientation behavior is significantly altered after exposure to a brief, strong magnetic pulse (Ernst and Lohmann, 2016), a stimulus known to affect magnetic orientation in a number of animals (Kirschvink, et al., 2001). Moreover, previous work found evidence for permanently magnetic material in the spiny lobster, consistent with the hypothesis that its magnetoreceptors are based on iron-oxide nanocrystals, such as magnetite (Lohmann, 1984). These findings highlight the potential of the spiny lobster for elucidating the genes and pathways that mediate magnetic field detection. Until recently, however, studies examining tissue-wide gene expression in this species have been difficult and cost-prohibitive.

In this study, I exposed lobsters to two different treatments: (1) a strong magnetic pulse (treatment); or (2) a sham pulse, in which lobsters were handled identically but not exposed to a pulse (control). A central nervous system transcriptome was then sequenced using the supraesophageal ganglion, subsophageal ganglion, and thoracic ganglia from all animals. This transcriptome will provide resources for future studies investigating the neural basis of magnetoreception, the effects of strong electromagnetic fields on neural physiology, comparative crustacean genomics, and molecular mechanisms in the crustacean central nervous system.

Data description

Animal collection and maintenance

Caribbean spiny lobsters were collected from hard-bottom habitat by divers in the vicinity of Long Key, FL, USA (24.80^0N, 80.87^0W) using SCUBA and handheld nets. The collection of lobsters was authorized by the Florida Fish and Wildlife Conservation

Commission (permit SAL-14-1333-SR). Lobsters (*n*=8) were shipped overnight to the Neuroscience Institute at Georgia State University where they were maintained in two 800 L communal, plastic tanks filled with filtered and aerated artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH, USA). Both tanks shared recirculated seawater and maintained lobsters under the following controlled conditions: (1) 24-28^{0}C; (2) 32-35 ppt salinity; (3) 13h:11h light:dark cycle. All lobsters were fed shrimp and squid every other day and were held in these conditions for 9-10 days before treatment and tissue dissection. On the day prior to treatment, each lobster was removed from its tank, sexed, weighed, measured, and tagged by attaching a labeled zip tie to the right antenna. In addition, a small portion of an intact pleopod was carefully removed from each animal with scissors and examined under a microscope for determination of molt stage following procedures outlined in Lyle and MacDonald (1983) and Turnbull (1989). MIxS data on the collected specimens can be found in Table 4.1.

Treatment and tissue extraction

Prior to nervous tissue extraction, each lobster was temporarily removed from its holding tank for treatment. Five of the lobsters were subjected to a brief, strong magnetic pulse (duration=5 ms; intensity=85 mT) directed antiparallel to the horizontal component of the geomagnetic field, as described previously (Ernst and Lohmann, 2016). Three other lobsters were handled identically but not subjected to a magnetic pulse. All animals were returned to their tanks immediately after treatment for approximately 2.5 hours (143.5 ± 5.0 min).

After the 2.5-hour post-treatment period, lobsters were anesthetized on ice for 20 minutes (17.88 ± 1.96 min) in preparation for dissection and nervous tissue extraction. Three

tissue types were collected from each animal: (1) the supraesophagael ganglion (i.e., brain); (2) subesophageal ganglion; and (3) thoracic ganglia. After extraction, each tissue was immediately placed into sterile, RNase-free 5 ml Biopur ™ microcentrifuge tubes (Eppendorf North America, Hauppauge, NY, USA) filled with 4 ml of RNAlater (Qiagen Co., Valencia, CA, USA). All samples were then incubated at 4^0C overnight and frozen at -80^0C for future processing. The preserved tissue samples were transported on dry ice to the Genomic Sciences Laboratory at North Carolina State University (Raleigh, NC, USA) for RNA isolation, library preparation, and sequencing.

RNA isolation, library preparation, and Illumina sequencing

Total RNA extraction was performed with the RNeasy Lipid Tissue Mini Kit (Qiagen Co., Valencia, CA, USA), and RNA quality was assessed using a 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA libraries were prepared using the Ultra Directional RNA Prep Kit for Illumina (New England BioLabs Inc., Ipswich, MA, USA). All libraries underwent quality assessment using a Tapestation 2200 (Agilent Technologies, Santa Clara, CA, USA) prior to sequencing. Libraries were then subjected to 75 bp paired-end (PE) sequencing on two lanes of a NextSeq 500 (Illumina Inc., San Diego, CA, USA). A total of >1.9 billion reads (>978 million 75 bp PE reads) were generated (Table 4.2).

Trimmomatic v0.36 (Bolger et al., 2014) was used to remove Illumina adapter sequences from each read. In addition, the leading and trailing ends of each read were trimmed to a minimum phred-scaled quality score (Q) ≥20. Then, using a sliding window of four bases, we further removed the trailing base if the mean quality score was <20. After trimming, reads shorter than 50 bp were removed. A total of >1.5 billion high quality reads

(>790 PE reads; 80.9% of the raw sequenced reads; Table 4.2) remained for transcriptome assembly.

De novo transcriptome assembly

Trinity v2.20 (Grabherr et al., 2011) was used to assemble the trimmed reads using a minimum contig length of 200 bp. A total of 298804172 bp were assembled into 327116 transcripts (mean = 913.45 bp; N50 = 1945 bp; Table 4.2). This raw assembly was then BLASTed against the UniVec database (http://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/), and two transcripts identified as likely vector contaminants (TRINITY_DN132837_c0_g1_i1 and TRINITY_DN36292_c0_g1_i1; vector=Cloning vector pBR322) were removed. All reads were then mapped back to the transcriptome to quantify expression using Bowtie2 (Langmead and Salzberg, 2012) and RSEM v1.2.31 (Li and Dewey, 2011) via the "align_and_estimate_abundance.pl" script included with the Trinity software, and the resulting abundance estimates were converted to a TPM (transcripts per million) matrix using the "abundance_estimates_to_matrix.pl" script.

After transcript quantification, an E90N50 statistic of 2850 bp was calculated for the assembly using the "contig_ExN50_statistic.pl" script (Table 4.2). All transcripts with a TPM <1 (149849 transcripts) were then removed using the "filter_low_expr_transcripts.pl" script, as there is little evidence supporting these transcripts (i.e., they are likely a result of error or background noise), and their contribution to overall expression is negligible.

To obtain the final transcriptome assembly, TransDecoder v3.0.1 (http://transdecoder.github.io/) was used to identify all open reading frames (ORFs) that were >100 amino acids in length (63083 ORFs), and the longest ORF for each isoform was

retained (30414 transcripts; Table 4.3). I then used CEGMA v2.4 (Core Eukaryotic Genes Mapping Approach; Parra et al., 2007) and BUSCO v3.0 (Benchmarking Universal Single-Copy Orthologs; Simao et al., 2015) to evaluate the completeness of the *de novo* assembly. Of the 248 CEGMA conserved core eukaryotic gene dataset, 233 complete genes and 4 gene fragments were present in the final assembly, accounting for 95.6% of the core eukaryotic genes. Furthermore, BUSCO analyses found that a high percentage of genes from the eukaryota (94.7%), metazoan (91.9%), and arthropoda (91.2%) databases were present in the *de novo* transcriptome.

Functional annotation and GO classification

I used Blast2GO v4.1.9 (Conesa et al., 2005) to complete the functional annotation and gene ontology (GO) classification of the 30414 transcripts comprising the *de novo* assembly. First, BLASTx v2.2.30+ (Altschul et al., 1990) was used to search the NCBI non-redundant ('nr') protein database (www.ncbi.nlm.nih.gov/refseq), and the top 10 hits with an e-value cut-off of 10^{-3} were retained. In addition, InterProScan v5.22-61.0 (Jones et al., 2014) was used to further identify and annotate transcripts via functional classification of proteins in the assembly. All BLASTx and InterProScan results were then uploaded into Blast2GO, and the default parameters were used for the remaining 'Mapping' and 'Annotation' steps to complete the functional annotation. All results were saved and exported as GO terms (www.geneontology.org).

The species with the highest number of BLAST top-hits were the amphipod *Hyalella azteca* (8809 hits), the termite *Zootermopsis nevadensis* (1322 hits), and the horseshoe crab *Limulus polyphemus* (713 hits). Of the GO terms present in the final assembly, 30912 were

associated with biological processes, 25720 with cellular components, and 20865 with molecular functions (Fig. 4.1).

Data deposition

The raw sequence data, transcriptome assembly, and functional annotation data from this study are available from the author upon request.

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Tables

Table 4.1: MIxS descriptors for the study.

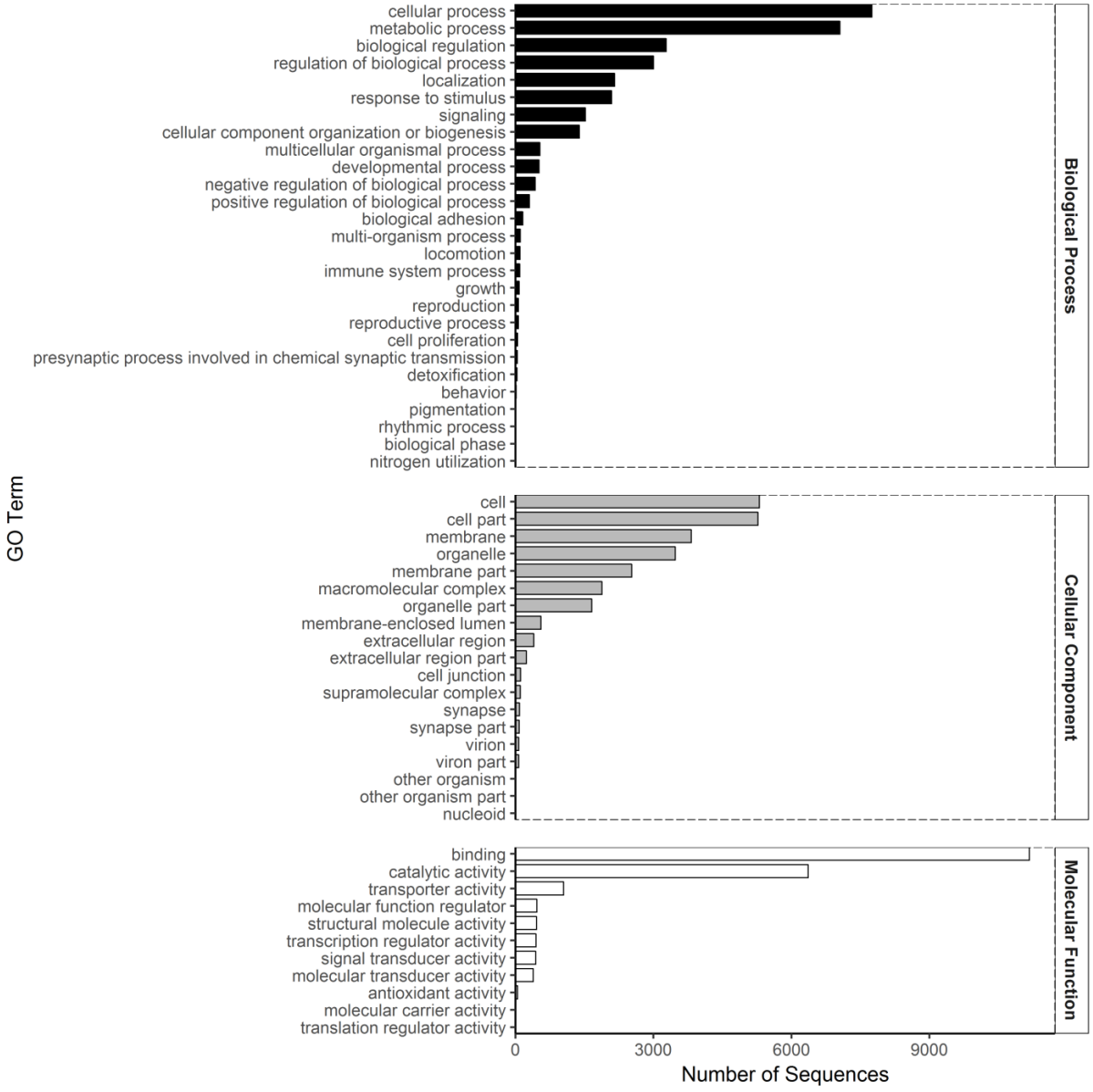
Item	Description
Investigation_type	Eukaryote
Project_name	*Panulirus argus* CNS transcriptome
Lat_lon	24.797217 N 80.867133 W
Geo_loc_name	USA: Long Key, Florida
Collection_date	10-Aug-2016
Biome	marine biome (ENVO:00000447)
Feature	marine benthic biome (ENVO:01000024)
Material	sea water (ENVO:00002149)
Env_package	Water
Seq_meth	Illumina NextSeq 500
Assembly	Trinity v2.20

Table 4.2: RNA-seq and transcriptome assembly statistics. PE=paired end; bp=base pairs; ORFs=open reading frames.

Metric	Statistic
Total raw reads (PE)	978327342
Reads post-trimming (PE)	790978119
Mapped reads	712955728 (90.1%)
Total assembled bases	298804172
Total Trinity 'genes'	242063
Total Trinity transcripts	327116
Median contig length (bp)	395
Average contig length (bp)	913.5
Maximum contig length (bp)	20624
Minimum contig length (bp)	201
GC content	40.8%
N50 (bp)	1945
E90N50 (bp)	2850
Transcripts with TPM >1	177265
Transcripts (longest ORFs) retained in final assembly	30414(15150 ‘genes’)
Annotated transcripts in final assembly	17912 (58.9%)

Figures

Figure 4.1: Gene ontology term distribution for the *de novo* transcriptome assembly.



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CHAPTER V

THE EFFECTS OF A MAGNETIC PULSE ON THE SPINY LOBSTER CENTRAL NERVOUS SYSTEM: TRANSCRIPTOMIC INSIGHTS FOR MAGNETORECEPTION MECHANISMS

Introduction

Magnetoreception, or the ability to detect Earth's magnetic field, has mystified biologists for several decades. Numerous animals take advantage of this unique sensory modality for orientation and navigation over long and short distances, but little is known about how magnetic field perception is accomplished (Wiltschko and Wiltschko, 2005; Johnsen and Lohmann, 2005). Although several possible mechanisms have been proposed, two hypotheses have garnered the most evidence: (1) the radical pairs hypothesis, which proposes that the geomagnetic field is detected through a complex series of light-induced chemical reactions that are sensitive to weak magnetic fields, possibly involving cryptochromes (Ritz et al., 2000; Maeda et al., 2008; Liedvogel and Mouritsen, 2010); and (2) the magnetite hypothesis, which posits that the physical basis of the magnetic sense is biogenic nanocrystals of the ferrimagnetic mineral magnetite (Fe_3O_4), and information about Earth's field is transduced through the torque exerted on these crystals by the ambient field and mechanical activation of ion channels or secondary receptors (Kirschvink and Gould, 1981; Johnsen and Lohmann, 2005; Winklhofer and Kirschvink, 2010).

One technique that has been used extensively as a 'diagnostic test' of magnetite-based magnetoreception is subjecting animals to a brief, strong magnetic pulse and analyzing their

subsequent orientation behavior (Kirschvink et al., 2001; Shaw et al., 2015). Pulse magnetization, in principle, might remagnetize or disrupt magnetite crystals associated with magnetoreceptors, or possibly compromise the crystals' connection to the nervous system, resulting in altered perception of geomagnetic information (Kirschvink, 1983; Kirschvink et al., 1985). Indeed, previous studies have demonstrated significant impacts of a magnetic pulse on the orientation behavior of many species (Beason et al., 1995; Marhold et al., 1997; Wiltschko et al., 1998, 2002; Irwin and Lohmann, 2005; Holland et al., 2008; Holland, 2010; Holland and Helm, 2013; Ernst and Lohmann, 2016), with effects including induced orientation, minor shifts in migratory headings, and complete disruption of orientation. Nevertheless, these studies only analyzed the behavioral effects of pulse magnetization on animal orientation and navigation, leaving the physiological and molecular effects of the pulse unclear.

Recent research on the rainbow trout (*Oncorhynchus mykiss*), a species known to respond to magnetic fields (Walker et al., 1997; Haugh and Walker, 1998; Hellinger and Hoffmann, 2009, 2012), revealed that a magnetic pulse elicited significant changes in gene expression in the brain, with 181 genes showing altered expression compared to control animals (Fitak et al., 2017). Included in this gene set were six copies of the *frim* gene, which encodes a subunit of the iron-binding and storage protein ferritin, and numerous genes linked to oxidative stress and photosensitive structures. Further analysis of reads that did not map to the trout genome revealed 12 additional genes with altered expression, including superoxide dismutase, a protein that prevents oxidative damage induced by reactive oxygen species, and collagen alpha-1 type II, a protein associated with retinal development and structural integrity (Arniella et al., 2018). Moreover, gene expression analyses in the trout retina after

exposure to the same magnetic pulse treatment revealed that only a single gene encoding a structural component of the eye lens (*crygm3*) responded to the pulse, indicating that iron-based magnetoreceptors are likely not located in the retina of this species (Fitak et al., *in review*). While these results provide novel and exciting evidence for a potential effect on iron-based receptors in the brain and resulting oxidative consequences, whether a magnetic pulse elicits changes in trout orientation behavior is not known.

Of the diverse animals that have a magnetic sense, the Caribbean spiny lobster, *Panulirus argus*, is among the most promising for investigation of the neural basis of magnetoreception for several reasons. First, spiny lobsters possess both a magnetic 'compass' (Lohmann et al., 1995) and 'map' sense (Boles and Lohmann, 2003), making this lobster the only invertebrate species known to derive both directional and positional information from Earth's magnetic field (Lohmann and Ernst, 2014). Second, pulse magnetization treatments altered the orientation of lobsters, providing the first evidence that a magnetic pulse affects the behavior of an invertebrate animal (Ernst and Lohmann, 2016). Finally, SQUID magnetometry detected permanently magnetic material within the body of spiny lobsters, indicating potential concentrations of iron and, possibly, iron-based magnetoreceptors (Lohmann, 1984).

As an initial step toward identifying genes that are associated with magnetoreception in spiny lobsters and elucidating the neurophysiological effects of pulse magnetization, I examined gene expression throughout the lobster central nervous system in response to a strong magnetic pulse. Based on previous findings in rainbow trout, I predicted that if iron-based receptors are damaged or otherwise disrupted by the pulse, the expression of genes

associated with iron metabolism and oxidative stress responses should be altered in pulsed lobsters relative to controls.

Materials and Methods

Animals

Caribbean spiny lobsters were collected in July 2016 by divers near Long Key, FL, USA. They were then shipped to Georgia State University and subsequently maintained for 9-10 days in two 800 L communal, plastic tanks filled with filtered and aerated artificial seawater (32-35 ppt; Instant Ocean, Aquarium Systems, Mentor, OH, USA). Lobsters were fed every other day and maintained at 24-28°C under a 13h:11h light:dark cycle. The collection of lobsters was authorized by the Florida Fish and Wildlife Conservation Commission (permit SAL-14-1333-SR).

Magnetic pulse treatment

After lobsters were weighed, measured, and sexed, the molt stage was determined using the protocols in Lyle and MacDonald (1983) and Turnbull (1989) (Table 5.1). Lobsters were then divided into two groups. Individuals from each group were subjected to one of two treatments: (1) a magnetic pulse (duration=5 ms; intensity=85 mT) directed antiparallel to the horizontal component of the geomagnetic field (n=4; Pulsed; Fig. 5.1A), as previously described (Ernst and Lohmann, 2016); or (2) a sham pulse, in which lobsters were handled identically but not subjected to a magnetic pulse (n=3; Control; Fig. 5.1B). Animals were placed back into their tanks after treatment for a 2.5 hr (143.5 ± 5.0 min) period prior to nervous tissue extraction. The order of treatment was interspersed over the course of two days (Table 5.1).

After the 2.5 hr period, each lobster was removed from its tank, placed in a plastic container, and immediately anesthetized on ice for a period of 20 min (17.88 ± 1.96 min). The supraesophagael ganglion (i.e., brain), subesophageal ganglion, and thoracic ganglia were then removed. Each ganglion was separately preserved in RNAlater (Qiagen Co., Valencia, CA, USA) and stored at -80^{0}C until processing.

RNA extraction, sequencing, and quality trimming

RNA extraction, library preparation, and RNA sequencing were completed by the Genomic Sciences Laboratory at North Carolina State University (Raleigh, NC, USA); methods are described in detail in Chapter IV. Briefly, a total of ~978 million 75 bp paired-end reads were sequenced on two lanes of a NextSeq 500 (Illumina Inc., San Diego, CA, USA; Table 5.2). All reads were subsequently quality trimmed using Trimmomatic v0.36 (Bolger et al., 2014), resulting in >790 million high quality paired-end reads (80.9% of the raw sequenced reads).

Differential gene expression analysis

To quantify expression, all trimmed reads from the pulsed and control groups were mapped to a *P. argus* central nervous system reference transcriptome (see Chapter IV) using Bowtie2 (Langmead and Salzberg, 2012) and RSEM v1.2.31 (Li and Dewey, 2011) via the "align_and_estimate_abundance.pl" script included with the Trinity v2.20 (Grabherr et al., 2011) software (Table 5.2). To analyze differential gene expression in each of the three tissues, I used the DESeq2 v1.16.1 package (Love et al., 2014) in R (Version 3.3.3, R Foundation for Statistical Computing, Vienna, Austria), which allowed me to fit a generalized linear model to each gene. I used the model:

$$y \sim treatment\ day + sex + molt\ stage + group$$

where *group* represents a grouping variable that combines tissue and treatment (e.g., Brain _Pulsed, Brain_Control, etc.). This design allowed me to contrast the effects of the magnetic pulse treatment at the gene level for each of the three tissues separately while controlling for differences in gene expression due to *treatment day* (08/10/16 or 08/11/16), *sex* (male or female), and *molt stage* (premolt, intermolt, or postmolt). Differences between groups were calculated as the binary log of the expression ratio of pulsed tissue groups relative to control tissue groups (log_2FC). Only genes with a false discovery rate <0.05 (FDR; Benjamini and Hochberg, 1995) were considered differentially expressed and retained for further analysis.

Gene ontology enrichment analyses

TopGO v2.28.0 (Alexa and Rahnenfuhrer, 2016) was used to test for gene ontology (GO) term enrichment in each of the three tissues using the Fisher's exact test and the 'classic' algorithm. Because Trinity assembles both isoforms (i.e., individual transcripts) and genes (i.e., clusters of transcripts with shared sequence but different structure, likely representing alternative splice variants or paralogs), GOs from all isoforms were combined for a given gene, and redundant GOs were removed to eliminate bias during analysis. To avoid potential biases from rare GOs, terms with fewer than 5 occurrences in the dataset were excluded from analysis. Only GO terms that were significant after correcting for multiple comparisons (FDR < 0.05; Benjamini & Hochberg 1995) were retained. To further explore GO enrichment across the nervous system in response to the pulse treatment, GOExpress v1.10.0 (Rue-Albrecht et al., 2016) was used. This analysis package uses machine learning

methods that employ a random forest statistical framework to identify GO terms associated with genes that best classify the samples based on gene expression profiles. After surveying 1000 trees, only GO terms that were significant ($P<0.05$) and associated with ≥5 genes after 10000 permutations were retained.

Results

Altered genes across all tissues

A large number of genes showed altered expression in response to the magnetic pulse treatment, with 647 genes, 1256 genes, and 712 genes differentially expressed in the brain, subesophageal ganglion, and thoracic ganglia, respectively (FDR < 0.05; Fig. 5.2). A cross-comparison of all differentially expressed genes (DEGs) revealed that 383 (17.2% of all unique DEGs) genes were present across all tissues (Fig. 5.3A). A total of 107 (16.5%) brain DEGs were unique to the brain, 629 (50.1%) subesophageal ganglion DEGs were unique to the subesophageal ganglion, and 118 (16.6%) thoracic ganglia DEGs were unique to the thoracic ganglia (Fig. 5.3A).

GO enrichment analysis found that 13 GO terms were significantly enriched (FDR<0.05) in the brain, 57 in the subesophageal ganglion, and 6 in the thoracic ganglia (Table 5.3; Appendix 5.1-5.3). Of these, 3 GO terms were shared between the brain and thoracic ganglia (hydrolase activity hydrolyzing O-glycosyl compounds, hydrolase activity acting on glycosyl bonds, and carbohydrate binding). In addition, 2 terms were shared between the brain and subesophageal ganglia (oxidoreductase activity and electron carrier activity; Fig. 5.3B). Tissue-independent GO analyses revealed that 70 (1.7%) of the 4222 GO annotations assigned to the transcriptome were significantly associated with gene expression differences induced by the magnetic pulse treatment (Table 5.4; Appendix 5.4). The terms

with the highest ranks were telomere maintenance (*P*=0.002), ribosomal small subunit assembly (*P*=0.011), and glucose metabolic process (*P*=0.014).

The magnetic pulse affected the expression of numerous genes encoding proteins involved with iron metabolism in all three tissues. These proteins included transferrin/transferrin precursor (*TF*), solute carrier family 40 member 1-like (*SLC40A1*; ferroportin), and metalloreductase STEAP3/STEAP4-like (*STEAP3/4*), each of which are involved with iron ion transport and homeostasis (Liang et al., 1997; Donovan et al., 2005; Scarl et al., 2017). Furthermore, the expression of a gene encoding complex III assembly factor LYRM7 (*LYRM7*), a protein involved in binding and stabilizing iron-sulfur clusters in the mitochondrial matrix (Sánchez et al., 2013), was significantly altered in all tissues. Numerous other genes encoding proteins with functions linked to iron metabolism were also found to be differentially expressed in a tissue-dependent manner (see below).

A variety of other physiological processes were also affected by the pulse across all tissues, including those linked to immune processes, mitochondrial functions, stress response, DNA repair, and hormone metabolism. Several genes encoding proteins involved with the innate immune response, such as 3 copies of C-type lectin, numerous ficolin/fibrinogen-related proteins, 2 anti-lipopolysaccharide factors, caspase, and 2 beta-1,3-glucan-binding precursor proteins, showed altered expression relative to control tissues. Furthermore, genes indicative of oxidative and cellular stress responses were universally affected by the pulse treatment, including 2 copies of superoxide dismutase [Cu-Zn], as well as genes encoding IMPACT-like, crustacean hyperglycemic hormone, Class B secretin G-coupled receptor GPRmth5, and genes involved with DNA damage repair (DNA excision repair ERCC-1, probable E3 ubiquitin- ligase RNF144A, and activating signal cointegrator 1 complex

subunit 3-like). A combination of 5 copies of esterase E4-like/juvenile hormone esterase-like carboxylesterase 1/2, 2 copies of juvenile hormone-inducible, and the transcription factor Krueppel homolog 1-like also showed differences in expression, indicating an effect on juvenile hormone metabolism. In addition, the pulse altered the expression of several genes linked to phototransduction, including genes for carotenoid isomerooxygenase and retinol dehydrogenase 12-like, and a gene linked to circadian rhythms in *Drosophila* (rhythmically expressed gene 5, or *Reg-5*).

Effects on the brain

A total of 647 genes (consisting of 1818 isoforms) were differentially expressed in the brain in response to the magnetic pulse (FDR < 0.05; Fig. 5.2A). Of these, 352 (2.3%) showed significant increases in expression and 295 (1.9%) showed decreased expression. GO enrichment analyses revealed that these genes were significantly enriched for a total of 13 GO annotations (Table 5.3; Appendix 5.1), with the top being hydrolase activity, hydrolyzing O-glycosyl compounds (FDR = 0.0034), catalytic activity (FDR = 0.0034), and intrinsic component of membrane (FDR = 0.0092).

The annotated genes with the greatest differences in expression relative to controls include those encoding the proteins C-type lectin ($\log_2$FC = -2.86; FDR = 1.0×10^{-24}), 2 copies of esterase E4-like (copy 1: $\log_2$FC = 2.60; FDR = 5.0×10^{-13}; copy 2: $\log_2$FC = 2.59; FDR = 4.0×10^{-12}), and complex III assembly factor LYRM7 ($\log_2$FC = 2.53; FDR = 7.7×10^{-44}). Moreover, in addition to the numerous genes associated with iron metabolism that were differentially expressed in all tissues, expression of a gene encoding glutaredoxin-related protein 5 mitochondrial-like (*GLRX5*) was also altered in the brain. This protein is associated with the biogenesis of iron-sulfur clusters and iron homeostasis (Ye et al., 2010).

The differential expression of additional genes indicating oxidative stress was also seen in the brain, including those encoding the proteins glutathione peroxidase 3 and thioredoxin-dependent peroxide mitochondrial (Table 5.5). Furthermore, the expression of many genes associated with DNA repair (e.g., DNA repair RAD51 homolog 3, DNA damage-binding 2 isoform X1/2, and DNA damage-inducible transcript 4) was also altered (Table 5.6).

Effects on the subesophageal ganglion

The magnetic pulse altered the expression of 1256 genes (consisting of 3274 isoforms) in the subesophageal ganglion (FDR < 0.05; Fig. 5.2B). Among these, 701 (4.6%) had significantly elevated expression, and 555 (3.7%) had significantly decreased expression. GO enrichment analyses revealed that these genes were significantly enriched for a total of 57 GO annotations (Table 5.3; Appendix 5.2), many of which were associated with protein synthesis and mitochondrial functions. The top GO terms were structural constituent of ribosome (FDR = 1.2×10^{-19}), ribosome (FDR = 4.1×10^{-18}), and intracellular ribonucleoprotein complex (FDR = 6.0×10^{-13}).

The annotated genes with the largest differences in expression relative to controls were those encoding the proteins C-type lectin ($\log_2$FC = -3.13; FDR = 2.0×10^{-30}), esterase E4-like (log2FC = 2.78; FDR = 5.6×10^{-15}), and ficolin 2/fibrinogen-related 1 isoform 8/ficolin (collagen fibrinogen domain containing) 3 precursor ($\log_2$FC = -2.71; FDR = 1.7×10^{-18}). In addition, several other proteins involved with iron homeostasis were altered. These include additional copies of transferrin/pacifastin heavy chain precursor and solute carrier family 40 member 1-like, ZIP14-like (a protein that mediates cellular iron and zinc uptake; Liuzzi et al., 2006; Zhao et al., 2010), glutaredoxin-related protein 5 mitochondrial-like, and

heme oxygenase 1 (*HMOX1*; a protein that degrades heme to release ferrous iron and is also involved with oxidative stress responses; Kikuchi et al., 2005).

Numerous other genes associated with oxidative stress also showed significantly altered expression, including those encoding the proteins nucleoside diphosphate kinase homolog 5-like, peroxiredoxin 6, and metallothionein (Table 5.5), and several genes linked to DNA repair processes (e.g., DNA mismatch repair Msh6-like, apurinic apyrimidinic endonuclease apn1, and non-structural maintenance of chromosomes element 1 homolog; Table 5.6).

Effects on the thoracic ganglia

A total of 712 genes (consisting of 1879 isoforms) were differentially expressed in the thoracic ganglia in response to the magnetic pulse (FDR < 0.05; Fig. 5.2C). Of these, the expression of 315 (2.1%) was significantly increased and 397 (2.6%) was reduced. GO analyses revealed that these genes were significantly enriched for a total of 6 GO annotations (Table 5.3; Appendix 5.3), with the top being carbohydrate metabolic process (FDR = 0.0086), hydrolase activity, hydrolyzing O-glycosyl compounds (FDR = 0.010), and hydrolase activity, acting on glycosyl bonds (FDR = 0.011).

The most highly-altered annotated genes include those encoding the proteins C-type lectin ($\log_2$FC = -3.29; FDR = 6.2×10^{-33}), chitin deacetylase 1 precursor ($\log_2$FC = 2.72; FDR = 7.7×10^{-17}), and ficolin 2/fibrinogen-related 1 isoform 8/ficolin (collagen fibrinogen domain containing) 3 precursor ($\log_2$FC = -2.70; FDR = 2.7×10^{-18}). Similar to the brain and subesophageal ganglion, differences in the expression of additional genes involved with iron metabolism were observed, including those encoding an extra copy of transferrin/pacifastin heavy chain precursor, sideroflexin-2 (*SFXN2*), and cytoplasmic aconitate hydratase-like

isoform X1 (i.e., iron regulatory protein 1, *IRP1*; a protein critical to regulating iron levels; Huang et al., 1999).

Furthermore, genes encoding proteins associated with oxidative stress also showed altered expression in the thoracic ganglia of pulsed lobsters (e.g., phospholipid-hydroperoxide glutathione peroxidase, nucleoside diphosphate kinase homolog 5-like, and peroxidase-like isoform X2/chorion peroxidase; Table 5.5). In addition, several genes linked to DNA damage showed altered expression, including DNA helicase MCM8 isoform X2 and DNA damage-inducible transcript 4 (Table 5.6).

Discussion

Exposure to a brief, strong magnetic pulse elicited altered expression of a large number of genes throughout the spiny lobster central nervous system. Genes affected included ones involved with iron regulation, response to oxidative stress, DNA damage/repair, immune response, and phototransduction. These results are consistent with the magnetite hypothesis of magnetoreception, inasmuch as they are consistent with the interpretation that the pulse damaged or altered iron-based magnetoreceptors located in the lobster central nervous system. Furthermore, the number and diversity of genes altered by the pulse indicates that a magnetic pulse has a substantial impact on neural physiology, suggesting that the effects of a strong magnetic pulse are likely not exclusive to magnetoreceptors.

Effect on iron regulation

A magnetic pulse might affect iron-based magnetoreceptors through remagnetization (e.g., reversing the magnetic dipole moment of magnetite crystals) and/or physical damage to

the receptor as a result of translocation of iron particles upon exposure to the strong magnetic gradient produced by the pulse. Therefore, I predicted that if iron-based magnetoreceptors are located within the spiny lobster central nervous system, a magnetic pulse will induce the expression of genes involved with iron regulation, possibly indicating magnetoreceptor repair or replacement, the deleterious oxidative effects of free iron on cells (Winterbourn, 1995; Emerit et al., 2001), and processes related to tissue damage.

Differential expression analyses found that in each tissue, genes linked to iron binding, transport, and homeostasis showed significantly altered expression. Moreover, gene ontology analyses revealed the enrichment of GO terms associated with iron, including heme binding and inorganic cation transmembrane transporter activity (Appendix 5.1-5.4). Numerous enriched GOs were also associated with oxidative damage, including peroxidase activity, 4 GOs linked to oxidoreductase activities (oxidoreductase activity, oxidation-reduction process, protein disulfide oxidoreductase activity, and electron carrier activity), and 4 GOs indicating DNA damage and repair (cellular response to DNA damage stimulus, double-strand break repair via nonhomologous end joining, double-strand break repair, nucleotide-excision repair, and telomere maintenance). Effects on these processes indicate a disruption of iron homeostasis in the nervous system and possibly the subsequent oxidative consequences on cellular membranes, proteins, and DNA (Winterbourn, 1995; Emerit et al., 2001).

Three genes encoding proteins central to iron regulation were differentially expressed relative to controls across all tissues, including transferrin/transferrin precursor (*TF*), the metalloreductase STEAP3/STEAP4-like (*STEAP3/4*), and solute carrier family 40 member 1-like (*SLC40A1*; ferroportin). Transferrin is one of the most important iron transport proteins

in animals, in that it binds extracellular iron and transports it to various tissues to control cellular iron concentrations (Huebers et al., 1982; Aisen, 1998). When iron-bound transferrin encounters a transferrin receptor on a cell surface, it binds to the receptor to form a complex, enters the endosome of the cell, and the complex then releases the iron as Fe^{3+}. However, for iron to cross the endosomal membrane and enter the cell, it must be reduced to Fe^{2+} by *STEAP3*/*4*. Like transferrin, *STEAP3* and *STEAP4* are therefore critical to cellular iron homeostasis (Ohgami et al., 2006; Zhao et al., 2013; Scarl et al., 2017). By contrast, ferroportin regulates cellular iron concentrations by exporting iron out of cells (Donovan et al., 2005; Boserup et al., 2011; Ward and Kaplan, 2012). Pulse-induced expression changes in these genes suggest that they might be involved with the repair or replacement of damaged iron-based magnetoreceptors in the central nervous system through removing excess iron expelled from impaired receptors or supplying iron to cells for the biogenesis of new iron-oxide nanocrystals.

Interestingly, a gene encoding complex III assembly factor LYRM7 (*LYRM7*) was among the genes with the highest increase in expression relative to controls in all three tissues. This protein functions in binding and stabilizing Rieske iron-sulfur (2Fe-2S) clusters in the mitochondrial matrix (Cui et al., 2012; Sánchez et al., 2013). Similarly, another protein involved with mitochondrial iron-sulfur cluster biogenesis, glutaredoxin-related protein 5 mitochondrial-like (*GLRX5*; Ye et al., 2010), showed increased expression in the brain and subesophageal ganglion. Although I cannot say for certain from the current study, it is possible that the pulse might have liberated iron-sulfur clusters within the mitochondrial matrix, possibly through damage to the various iron-dependent protein complexes of the electron transport chain, such as complex III. As a result, *LYRM7* and *GLRX5* expression

might have increased to bind, sequester, and/or stabilize these unbound clusters and reduce oxidative damage.

Other proteins also involved with iron regulation were differentially expressed on a tissue-dependent basis. In the brain and subesophageal ganglion, the pulse affected the expression of a gene encoding hypoxia inducible factor 1 alpha, a transcription factor that, among other things, regulates the expression of genes related to iron uptake and storage, including ferritin and the iron transporter *SMF3* (Romney et al., 2011). In the subesophageal ganglion, the pulse also altered the expression of zinc transporter ZIP14-like, a metal transporter that mediates iron uptake into cells (Liuzzi et al., 2006; Zhao et al., 2010). Furthermore, additional copies of genes encoding transferrin/pacifastin heavy chain precursor, a protein with 3 transferrin-like lobes (Liang et al., 1999), also showed altered expression in both the subesophageal ganglion and thoracic ganglia, potentially indicating a stronger effect on iron-bearing structures in these tissues. Additionally, cytoplasmic aconitate hydratase-like isoform X1 (iron regulatory protein 1; *IRP1*), a protein that acts as an 'iron sensor' and controls iron metabolism (Huang et al., 1999), was differentially expressed in the thoracic ganglia.

These results provide strong evidence that the magnetic pulse had an impact on iron homeostasis within the lobster central nervous system, possibly through the release of free iron as a result of damage to magnetoreceptors based on iron-oxides and subsequent repair or replacement of impaired receptors. Indeed, a number GO terms associated with iron-related oxidative stress, possibly as a result of elevated iron levels, were enriched in the central nervous system (Appendix 5.1-5.4). It is also possible that the magnetic pulse had a damaging effect on numerous proteins with iron cofactors which are unrelated to iron

regulation, resulting in altered expression to replace compromised proteins. Damage to these proteins might have resulted in unbound iron ions, further contributing to the oxidative stress activities observed throughout the nervous system. Further studies will be needed to confirm if the affected genes/proteins are linked to effects on magnetite-based magnetoreceptors in the lobster.

The results of the current study are remarkably similar to those seen in the trout brain, in which the expression of genes encoding subunits of the iron-storage protein ferritin and several proteins linked to oxidative stress was altered in response to a similar magnetic pulse (Fitak et al., 2017; Arniella et al., 2018). Although several ferritin homologs were present in the lobster central nervous system transcriptome (Chapter V), none of these genes were differentially expressed. Several key differences between the current work and trout studies might explain this discrepancy. First, while the pulse treatment used in the two studies was identical in duration and strength, the magnetic pulse orientations were in opposing directions relative to the ambient magnetic field (i.e., directed toward magnetic north in the trout work and directed toward magnetic south in the current study). Second, the animals in each study were euthanized at two very different time points (i.e., trout were euthanized ~5 minutes after pulse exposure, while lobsters were euthanized ~2.5 hours after pulse exposure). Finally, invertebrates and vertebrates often differ considerably in many aspects of physiology; for instance, spiny lobsters depend on copper-based proteins (hemocyanin) for oxygen transport, rather than the iron-based proteins (hemoglobin) that trout and other vertebrates utilize. Nevertheless, the similarities between the findings of the two studies are intriguing and offer an exciting comparative view of the effects of a magnetic pulse on putative magnetoreceptors and neurophysiology in disparate species.

Effect on visual proteins

The magnetic pulse affected the expression of multiple genes linked to photoreception, including several that encode carotenoid isomerooxygenase, which is involved in the extra-retinal biogenesis of the visual chromophore rhodopsin in *Drosophila* (Gu et al., 2004; Wang et al., 2007). In addition, the expression of genes encoding retinol dehydrogenase 12 was also affected. This protein is known to convert 11-*cis*-retinol to 11-*cis*-retinal in vertebrate photoreceptors (Thompson et al., 2005; Madea et al., 2006) and shows similar activity in *Drosophila* (Wang et al., 2010). Interestingly, genes associated with visual structures were also altered in response to a magnetic pulse in the trout brain (Fitak et al., 2017; Arniella et al., 2018). It is unclear, however, why the magnetic pulse affected the expression of genes associated with phototransduction pathways in the lobster nervous system.

One possibility is that the magnetic pulse triggered a response in or damaged extraocular photoreceptors located in the lobster central nervous system. Photosensitivity in the arthropod nervous system is widespread; for example, photoreceptors are located in the brains (Sandeman et al., 1990; Bobkova et al., 2003) and terminal abdominal ganglia of numerous crustaceans (Prosser, 1934; Wilkens and Larimer, 1976). In addition, opsins have been localized throughout the crayfish central nervous system (Kingston and Cronin, 2015), and evidence for photosensitivity exists in the central nervous system of *Limulus* (Mori and Kuramoto, 2004). In the current study, we also found numerous opsins in the spiny lobster central nervous system transcriptome (i.e., opsin Rh2; rhodopsin-like; rhodopsin, partial; 2 long wavelength-sensitive opsins; and compound eye opsin BCRH2), suggesting that phototransduction might exist in the lobster nervous system. It is also possible that the

magnetic pulse affected carotenoid isomerooxygenase proteins indirectly through the pulse's effects on iron metabolism, as these proteins bind Fe^{2+} cofactors (Kloer et al., 2005). Nevertheless, further studies are required to confirm that these proteins are indeed involved with photoreception in the lobster nervous system, as well as to elucidate the mechanism by which the expression of these proteins is altered by a magnetic pulse.

It is intriguing to note that none of the genes encoding cryptochromes in the lobster central nervous system transcriptome (i.e., 3 copies of cryptochrome 1 and 2 copies of cryptochrome DASH-like), proteins hypothesized to mediate chemical magnetoreception based on radical pairs (Liedvogel and Mouritsen, 2010), showed altered expression in any of the three lobster nervous tissues. While this does not rule out the radical pairs mechanism as a possible transduction pathway for magnetoreception in the lobster central nervous system, it does suggest that effects of a magnetic pulse on cryptochrome gene expression, if any, are likely short-lived (<2.5 hours) or have a long latency (>2.5 hours). Future time-series experiments, however, are needed to further elucidate potential effects on cryptochromes.

Other effects on gene expression

While the magnetic pulse had apparent effects consistent with an effect on iron-related processes in the central nervous system, a number of other processes were also affected in each tissue. Interestingly, unlike the brain and thoracic ganglia GO enrichment profiles, many of the GO terms enriched in the subesophageal ganglion were associated with protein synthesis (including ribosome, structural constituent of ribosome, ribosomal subunit, small ribosomal subunit, large ribosomal subunit, cytosolic ribosome, cytosolic small ribosomal subunit, cytosolic large ribosomal subunit, translation, organellar ribosome, and rRNA binding). In addition, a number of GO terms were also linked to mitochondria

(including proton-transporting ATP synthase complex coupling factor F(o), electron carrier activity, mitochondrial part, mitochondrial inner membrane, proton-transporting two-sector ATPase complex proton-transporting domain, mitochondrial envelope, mitochondrial membrane, mitochondrion, mitochondrial ribosome, aerobic respiration, proton-transporting ATP synthase complex, and tricarboxylic acid cycle) (Table 5.3; Appendix 5.2). Although the exact cause of such an effect on protein synthesis and mitochondrial functions in the subesophageal ganglion cannot be determined from the current study, these results might indicate a stress response induced by the magnetic pulse treatment in this tissue.

The magnetic pulse also elicited changes in the expression of genes related to an innate immune response. For instance, the pulse treatment altered the expression of genes encoding proteins with antimicrobial properties, such as C-type lectins (Jin et al., 2013; Zhang et al., 2016; Pees et al., 2016), anti-lipopolysaccharide factors (Tanaka et al., 1982; Beale et al., 2008; de la Vega et al., 2008), beta-1,3-glucan-binding precursor (Barracco et al., 1991; Thörnqvist et al., 1994; Vargas-Albores and Yepiz-Plascencia, 2000), ficolin/fibrinogen-like proteins (Söderhäll et al., 2009; Chai et al., 2012; Sun et al., 2014; Dai et al., 2017), scavenger receptor proteins (Rämet et al., 2001; Yang et al., 2016), and Limulus clotting factor C-like (Nakamura et al., 1986). In addition, prophenoloxidase activating factor, a protein that activates the invertebrate innate immune defense system in response to tissue damage and pathogens (Cerenius and Söderhäll, 2004), was differentially expressed in the subesophageal ganglion. Furthermore, several copies of esterase E4-like/juvenile hormone esterase-like carboxylesterase 1 also showed altered expression, suggesting an effect on juvenile hormone metabolism. Aside from its functions in molting, metamorphosis,

and reproduction (Homola and Chang, 1997), juvenile hormone is also linked to regulation of the innate immune system (Flatt et al., 2008; Tian et al, 2010; Schwenke and Lazzaro, 2017).

Although it is unclear why immune processes were affected, it is possible that the expression of these genes was altered as a result of damaged magnetite-based magnetoreceptors or other iron-based structures and the potential translocation of iron crystals within the central nervous system, leading to neural lesions and neural tissue damage. Additionally or alternatively, changes in the expression of genes associated with an immune response might be a result of links between microbial infection and iron-sequestering mechanisms employed by the innate immune system (Ong et al., 2006; Toe et al., 2012).

Interestingly, the magnetic pulse affected the expression of two genes encoding ion channels associated with sensory transduction, the transient receptor potential (TRP) channel homologs Pyrexia and TRPA1 in the thoracic and subesophageal ganglion, respectively. While both Pyrexia and TRPA1 are sensitive to temperature (Lee et al., 2005; Hamada et al., 2008), TRPA1 also plays sensory roles in the detection of pain, tissue damage, and reactive oxygen species in animals (Viana, 2016; Arenas et al., 2017). One possible interpretation of these findings is that they reflect pulse-induced tissue damage in the lobster subesophageal ganglion. Furthermore, TRPA1 is involved in mechanotransduction in *C. elegans* (Kindt et al., 2007). The magnetite-based magnetoreceptor model proposes that torque on magnetite crystals might be transduced through mechanically-activated ion channels (Johnsen and Lohmann, 2005). Thus, an interesting speculation is that TRPA1 might function in the mechanical transduction of magnetic information in the lobster nervous system, although future studies will be needed to confirm or refute this hypothesis.

Electric field effects

Although the treatment used in this experiment was designed to subject lobsters to a brief, strong magnetic pulse stimulus, it is possible that at least some of the observed effects on the lobster central nervous system were not caused by the magnetic field, but instead by electric field transients induced by the pulsed magnetic field. In accordance with Faraday's law, a changing magnetic field induces an electric field (Purcell, 1985); therefore, it is possible that the electrical transients induced by the pulse stimulus might have had additional effects on the central nervous system, potentially by stimulating neurons (Pashut et al., 2011) or altering electrochemical gradients. Though the pulse treatment clearly affected various functions in the central nervous system, more work is required to determine if these effects are specific to magnetic or associated electric fields.

Conclusions

This study is the first to demonstrate the effects of a magnetic pulse on gene expression in the central nervous system of an invertebrate. My results indicate that numerous genes involved with iron homeostasis, oxidative stress, immune response, and DNA repair show altered expression in response to the pulse treatment, consistent with the hypothesis that a pulse damages or disrupts iron-based magnetoreceptors in the lobster nervous system. Moreover, the pulse elicited differential expression of a large number of genes associated with a variety of biological functions, indicating a substantial effect on neural function. Further work is needed to determine the functional significance of the observed expression patterns in each tissue and to confirm that the candidate genes mentioned in this study are in fact linked to magnetoreception.

While I can speculate about how and why a magnetic pulse altered the expression of such a diversity of genes, one conclusion is clear: a magnetic pulse has significant effects on neural physiology, many of which are likely not directly linked to a magnetic sense. This finding is of considerable importance, as much of the strongest evidence for magnetite-based magnetoreception in animals consists of behavioral studies using pulse magnetization under the assumption that a magnetic pulse will only exert its effects on biogenic magnetite and, thus, the magnetic sense (Kirschvink et al., 2001). My results show that a variety of molecular pathways are also affected by a pulse in the lobster central nervous system. While a magnetic pulse certainly might exert effects on iron-based receptors, my findings strongly suggest that caution must be taken when interpreting the results of pulse magnetization behavioral studies.

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Tables

Table 5.1: Summary of individual lobster characteristics. Date = treatment date; CL = carapace length.

ID	Treatment	Date	Sex	Weight (g)	CL (mm)	Molt Stage
1	Pulsed	08/11/16	Male	377	75	Intermolt
2	Pulsed	08/11/16	Female	357	73	Intermolt
3	Control	08/10/16	Male	300	70.5	Premolt
4	Control	08/11/16	Female	325	70.5	Postmolt
5	Pulsed	08/10/16	Male	293	72	Intermolt
6	Pulsed	08/11/16	Female	203	60	Premolt
7	Pulsed	08/10/16	Female	303	65	Premolt
8	Control	08/10/16	Female	216	61.5	Intermolt

Table 5.2: Summary statistics for raw sequence data by tissue. Mean Mapped Reads indicates the number of raw reads that mapped to the *de novo* transcriptome assembly from Chapter V.

Tissue	**n**	**Total Raw Reads ($\times 10^6$)**	**Mean Raw Reads ($\times 10^6$)**	**Mean Mapped Reads ($\times 10^6$)**
Brain	8	327.0	40.9 ± 3.6	18.1 ± 1.9
SG	8	331.3	41.4 ± 2.9	18.6 ± 1.2
TG	8	320.0	40.0 ± 3.2	17.6 ± 1.7
All Tissues	24	978.3	40.8 ± 3.1	18.1 ± 1.6

Table 5.3: List of top significantly enriched GO terms in each tissue. BP=Biological Process; MF=Molecular Function; CC=Cellular Component.

GO Term	Description	FDR	Category
	Brain		
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	3.4×10^{-3}	MF
GO:0003824	catalytic activity	3.4×10^{-3}	MF
GO:0031224	intrinsic component of membrane	9.2×10^{-3}	CC
GO:0016021	integral component of membrane	9.2×10^{-3}	CC
GO:0016491	oxidoreductase activity	1.0×10^{-2}	MF
GO:0030246	carbohydrate binding	1.0×10^{-2}	MF
GO:0009055	electron carrier activity	1.0×10^{-2}	MF
GO:0016798	hydrolase activity, acting on glycosyl bonds	1.0×10^{-2}	MF
GO:0044710	single-organism metabolic process	2.0×10^{-2}	BP
GO:0004559	alpha-mannosidase activity	2.8×10^{-2}	MF
	Subesophageal ganglion		
GO:0003735	structural constituent of ribosome	1.2×10^{-19}	MF
GO:0005840	ribosome	4.1×10^{-18}	CC
GO:0030529	intracellular ribonucleoprotein complex	6.0×10^{-13}	CC
GO:1990904	ribonucleoprotein complex	6.0×10^{-13}	CC
GO:0044391	ribosomal subunit	8.3×10^{-11}	CC
GO:0005198	structural molecule activity	5.5×10^{-9}	MF
GO:0006412	translation	6.7×10^{-8}	BP
GO:0043043	peptide biosynthetic process	6.7×10^{-8}	BP
GO:1901566	organonitrogen compound biosynthetic process	6.7×10^{-8}	BP
GO:0043604	amide biosynthetic process	1.6×10^{-7}	BP

	Thoracic ganglia		
GO:0005975	carbohydrate metabolic process	8.6×10^{-3}	BP
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	1.0×10^{-2}	MF
GO:0016798	hydrolase activity, acting on glycosyl bonds	1.1×10^{-2}	MF
GO:0030246	carbohydrate binding	1.8×10^{-2}	MF
GO:0008483	transaminase activity	1.8×10^{-2}	MF
GO:0016769	transferase activity, transferring nitrogenous groups	1.8×10^{-2}	MF

Table 5.4: List of top 10 ranked GO terms in response to the magnetic pulse treatment. Average rank and score are calculated means for all genes assigned to each GO term, indicating the power to differentiate between the Pulsed and Control groups. Count=number of genes assigned to GO term; BP=Biological Process; MF=Molecular Function; CC=Cellular Component.

GO Term	Average Rank	Average Score ($\times 10^3$)	Count	*P*-value	Description	Category
GO:0000723	2400.9	6.80927	7	0.002	telomere maintenance	BP
GO:0000028	2471.7	0.796006	6	0.011	ribosomal small subunit assembly	BP
GO:0006006	2497.6	1.952753	5	0.014	glucose metabolic process	BP
GO:0006816	2511.7	4.275594	6	0.009	calcium ion transport	BP
GO:0004888	2642.6	2.197129	9	<0.001	transmembrane signaling receptor activity	MF
GO:0003756	2678.4	1.189697	5	0.022	protein disulfide isomerase activity	MF
GO:0006890	2685.6	1.197983	5	0.015	retrograde vesicle-mediated transport, Golgi to ER	BP
GO:0006635	2691.8	1.33719	5	0.027	fatty acid beta-oxidation	BP
GO:0007030	2726.4	1.996556	5	0.02	Golgi organization	BP
GO:0005891	2743.0	0.975197	6	0.015	voltage-gated calcium channel complex	CC

Table 5.5: Genes associated with oxidative stress in each tissue.

Trinity Gene ID	Protein(s)
	Brain
TRINITY_DN103533_c0_g1	G-coupled receptor Mth2-like isoform X1
TRINITY_DN102497_c8_g1	glutaredoxin-1 isoform X3/LOC105347460 isoform X2/LOC101162407 isoform X2
TRINITY_DN117088_c0_g1	glutathione peroxidase 3
TRINITY_DN112835_c1_g1	heat shock 67B2-like
TRINITY_DN107132_c6_g1	hypoxia inducible factor 1 alpha
TRINITY_DN101460_c7_g1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6
TRINITY_DN116214_c1_g1	peroxidase-like isoform X2/chorion peroxidase
TRINITY_DN100245_c2_g2	thioredoxin-dependent peroxide mitochondrial
TRINITY_DN93768_c0_g1	X-box-binding 1-like
	Subesophageal ganglion
TRINITY_DN104819_c4_g1	G-coupled receptor Mth2 isoform X1
TRINITY_DN102497_c8_g1	glutaredoxin-1 isoform X3/LOC105347460 isoform X2/LOC101162407 isoform X2
TRINITY_DN97124_c2_g1	glutathione S-transferase
TRINITY_DN108821_c2_g1	heat shock
TRINITY_DN112835_c1_g1	heat shock 67B2-like
TRINITY_DN114568_c1_g1	heat shock cognate 70
TRINITY_DN101597_c1_g1	heme-binding 2-like
TRINITY_DN107132_c6_g1	hypoxia inducible factor 1 alpha
TRINITY_DN104446_c1_g1	metallothionein
TRINITY_DN110114_c3_g1	microsomal glutathione S-transferase 1
TRINITY_DN101460_c7_g1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6
TRINITY_DN106072_c3_g1	nucleoside diphosphate kinase homolog 5-like
TRINITY_DN116214_c1_g1	peroxidase-like isoform X2/chorion peroxidase

TRINITY_DN116256_c5_g1	peroxidasin/T-cell surface tactile-like isoform X2
TRINITY_DN104671_c5_g1	peroxiredoxin 6
TRINITY_DN111524_c2_g1	peroxisomal biogenesis factor 19
TRINITY_DN100245_c2_g2	thioredoxin-dependent peroxide mitochondrial
TRINITY_DN93768_c0_g1	X-box-binding 1-like
	Thoracic ganglia
TRINITY_DN114611_c7_g3	Heat shock 67B2
TRINITY_DN101159_c0_g1	heat shock 70
TRINITY_DN106072_c3_g1	nucleoside diphosphate kinase homolog 5-like
TRINITY_DN116256_c5_g1	peroxidasin/T-cell surface tactile-like isoform X2
TRINITY_DN111524_c2_g1	peroxisomal biogenesis factor 19
TRINITY_DN98627_c2_g2	phospholipid-hydroperoxide glutathione peroxidase
TRINITY_DN93768_c0_g1	X-box-binding 1-like

Table 5.6: Genes associated with DNA damage and repair in each tissue.

Trinity Gene ID	Protein(s)
	Brain
TRINITY_DN112733_c8_g3	DNA damage-binding 2 isoform X1/2
TRINITY_DN108334_c3_g1	DNA damage-inducible transcript 4
TRINITY_DN111533_c8_g3	DNA repair RAD51 homolog 3 isoform X2
TRINITY_DN110851_c8_g3	E3 ubiquitin-ligase RNF168-like
TRINITY_DN112216_c3_g1	non-structural maintenance of chromosomes element 1 homolog
TRINITY_DN111487_c2_g1	regulator of telomere elongation helicase 1 homolog
	Subesophageal ganglion
TRINITY_DN106597_c1_g2	apurinic apyrimidinic endonuclease apn1
TRINITY_DN99951_c1_g1	ATP-dependent DNA helicase Q1-like
TRINITY_DN117256_c3_g2	claspin isoform X2
TRINITY_DN113229_c1_g1	DNA mismatch repair Msh6-like
TRINITY_DN110167_c8_g1	E3 ubiquitin-ligase RNF8-like isoform X2
TRINITY_DN112073_c2_g1	flap endonuclease 1
TRINITY_DN115275_c6_g1	general transcription factor IIH subunit 4
TRINITY_DN112216_c3_g1	non-structural maintenance of chromosomes element 1 homolog
TRINITY_DN97324_c0_g1	replication A 14 kDa subunit-like
TRINITY_DN108375_c6_g3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD H box 1 homolog
TRINITY_DN100230_c0_g1	ubiquitin-conjugating enzyme E2 T
	Thoracic ganglia
TRINITY_DN108334_c3_g1	DNA damage-inducible transcript 4
TRINITY_DN111609_c2_g1	DNA helicase MCM8 isoform X2

TRINITY_DN110851_c8_g3	E3 ubiquitin- ligase RNF168-like
TRINITY_DN110167_c8_g1	E3 ubiquitin- ligase RNF8-like isoform X2
TRINITY_DN112073_c2_g1	flap endonuclease 1
TRINITY_DN111487_c2_g1	regulator of telomere elongation helicase 1 homolog
TRINITY_DN108375_c6_g3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD H box 1 homolog

Figures

Figure 5.1: Magnetic and sham pulse treatments. (A) Magnetic pulse treatment: lobsters were subjected to a magnetic pulse directed antiparallel to the horizontal component of the geomagnetic field (i.e. toward magnetic south). (B) Sham pulse treatment: lobsters were handled identically to those in the magnetic pulse treatment, but were not subjected to a magnetic pulse. The cylinder represents the solenoid of the magnetizer, while the arrow outside the solenoid indicates the direction of the magnetic pulse (N, north; S, south).

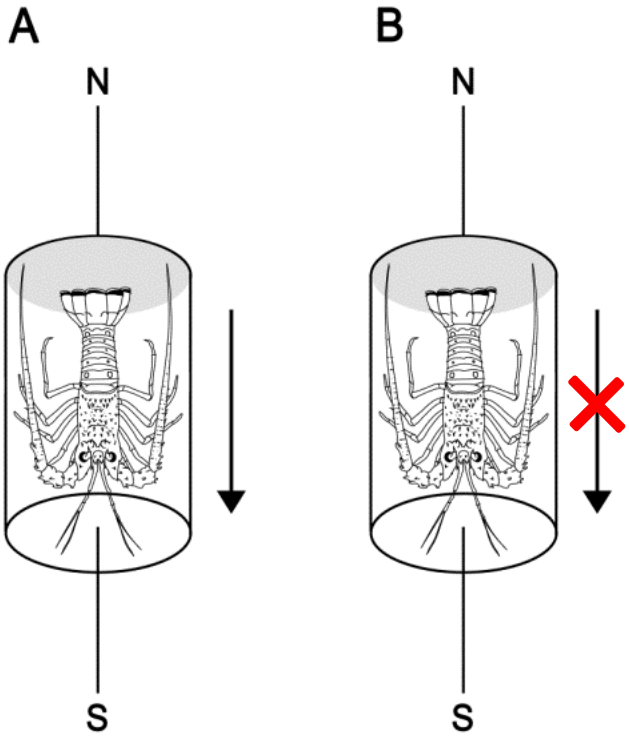


Figure 5.2: MA plots of the expression level (Mean of Normalized Counts) and ratio (log_2FC) for each gene in lobster tissues exposed to a magnetic pulse relative to control tissues. (A) MA plot of pulsed vs. control brains; (B) MA plot of pulsed vs. control subesophageal ganglia; (C) MA plot of pulsed vs. control thoracic ganglia. Differentially expressed genes are shown in red. A generalized additive model was fit to the data and is shown in blue. Genes linked to iron regulation are labeled (see text for details).

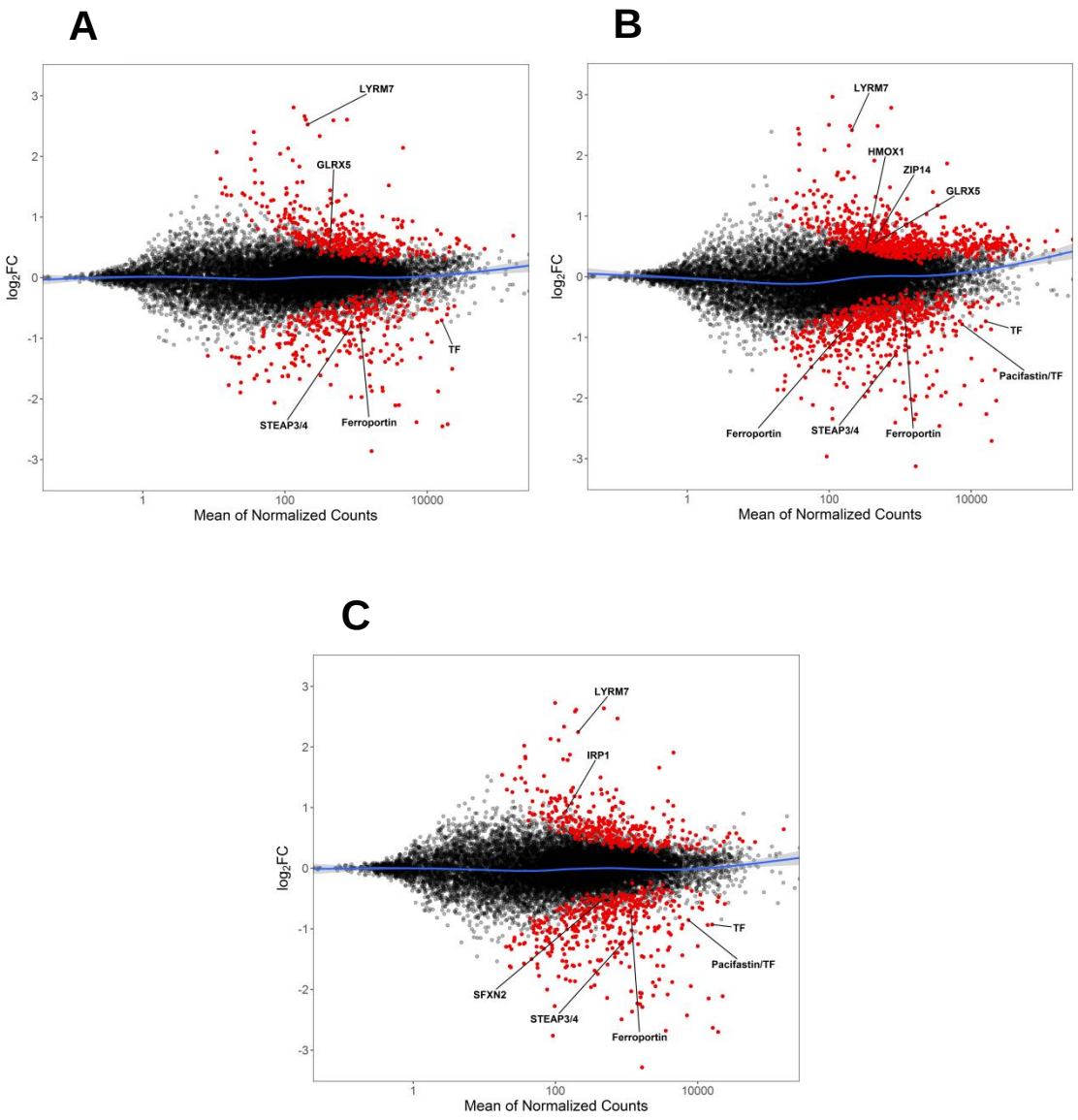
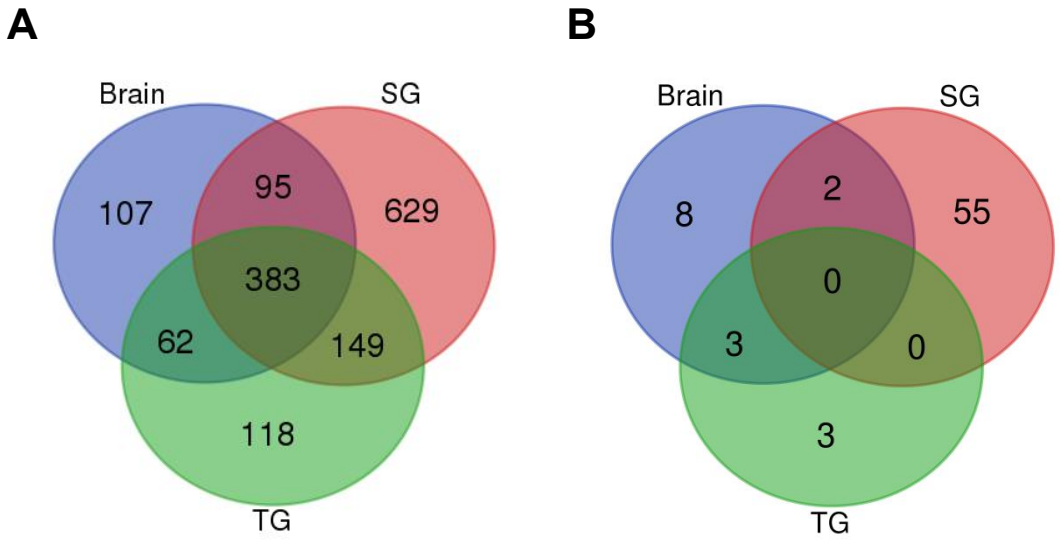


Figure 5.3: Venn diagrams showing the distributions of differentially expressed genes and GO terms across all tissues. (A) The relationship between differentially expressed genes across tissues. (B) The relationship between significantly enriched GO terms across tissues. SG=subesophageal ganglion; TG=thoracic ganglia.



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CHAPTER VI

CONCLUSIONS

The research described in this dissertation investigated magnetoreception by integrating behavioral and molecular approaches. The results provide novel insights into the effects of strong magnetic fields on lobster behavior and advance our understanding of magnetoreception and its underlying neural mechanisms. Moreover, these findings are likely to be relevant to other species that rely on a magnetic sense for orientation and navigation, especially those that are affected by pulse magnetization.

In my second chapter, I conducted an experiment testing the behavioral response of Caribbean spiny lobsters to a strong magnetic anomaly. Lobsters were allowed to choose between sheltering in artificial dens that were fitted with neodymium magnets or non-magnetic weights. Significantly more lobsters selected the control den, indicating avoidance of the magnetic anomaly, and lobster size was found to be a significant predictor of den choice. The mean size of lobsters that selected the anomaly den was significantly smaller than that of lobsters that chose the control den. These findings are consistent with previous work providing evidence for magnetoreception in spiny lobsters (Lohmann, 1985; Lohmann et al., 1995; Boles and Lohmann, 2003). Furthermore, the results suggest a possible ontogenetic shift in the spiny lobster's response to magnetic fields and indicate that magnetic anomalies have the potential to affect lobster movement patterns in the wild.

The finding that lobsters actively avoid strong magnetic anomalies is of considerable interest. Although the anomalies used in the work presented here were stronger than Earth's natural crustal anomalies, these results nonetheless provide initial evidence that localized magnetic fields can influence the spontaneous behavior of lobsters and possibly other animals. This effect also suggests that careful consideration is warranted in the construction and placement of anthropogenic structures that produce magnetic fields, such as oil rigs, submerged ship wrecks, wind turbines, and undersea cables. It is possible that these structures might have inadvertent effects on animal migrations, larval settlement, and/or local biodiversity through the production of unnatural magnetic stimuli.

It is interesting to note that many researchers in the field of magnetoreception have gone to great lengths to avoid subjecting animals to anomalous magnetic fields and unnatural field gradients, under the assumption that animals will not respond to magnetic fields unlike those that exist in the natural environment. Instead, researchers typically design and use carefully constructed magnetic coil systems to produce Earth-strength fields with high uniformity (Kirschvink, 1992). Given the results of the current study and previous behavioral studies that used magnets as a stimulus (Brown et al., 1960a,b; Thalau et al., 2007; Denzau et al., 2011; Freire et al., 2012; Kremers et al., 2014; O'Connell et al., 2015; Vidal-Gadea et al., 2015), it is interesting to note that subjecting animals to high intensity magnetic fields appears to be a useful technique for investigating magnetic sensitivity.

In addition, the finding that a lobster's size predicts its response to a magnetic anomaly is intriguing and deserves further investigation. Although we know that some animals such as sea turtles and birds have varying responses to magnetic fields over the course of their development (Lohmann and Lohmann, 2003; Lohmann et al., 2004; Denzau et

al., 2013; Munro et al., 2014), this area of research has generally received little attention. Understanding the differences in how animals respond to magnetic stimuli during various life history stages and why this variation is important will likely provide new insights into patterns of animal movement, such as dispersal and migration.

In my third chapter, I tested the 'magnetite hypothesis' of magnetoreception by subjecting lobsters to a strong magnetic pulse and examining subsequent orientation behavior. Lobsters were subjected to a single pulse directed either parallel or antiparallel to the horizontal component of the geomagnetic field (i.e., toward magnetic north or south, respectively), while a control group was subjected to a sham pulse. Control lobsters displayed random orientation as a group, but the two groups exposed to pulsed fields were significantly oriented in approximately opposite directions. These findings are consistent with magnetite-based magnetoreception in spiny lobsters.

Prior to this work, studies investigating the effect of a magnetic pulse on orientation behavior had been limited to vertebrate groups, such as birds (Beason et al., 1995; Wiltschko et al., 1998, 2002; Holland, 2010; Holland and Helm, 2013), turtles (Irwin and Lohmann, 2005), and bats (Holland et al., 2008). This study is the first to provide evidence that a magnetic pulse also affects the orientation behavior of an invertebrate species. This is an important finding, as it sets the stage for further investigation of the effects of pulse magnetization on invertebrates, a group that makes up >98% of all species on Earth (Pechenik, 2010). Moreover, it is curious that the magnetic pulse treatments induced orientation in lobsters. While it is unclear why pulsed lobsters exhibited directed orientation, it is possible that the pulse had an effect on receptors associated with the lobsters' magnetic map sense, causing lobsters to misinterpret their position relative to home. However,

additional experiments designed to test this possibility are needed to confirm or refute this hypothesis.

In my final chapters, I investigated the effect of a magnetic pulse on the lobster central nervous system, with a view toward identifying genes associated with magnetoreception and also identifying other possible physiological effects of the pulse. Specifically, I subjected lobsters to either a magnetic pulse oriented antiparallel to the geomagnetic field (as described in Chapter III) or a sham pulse. RNA sequencing was then used to assemble a *de novo* central nervous system transcriptome, and gene expression was examined in the brain, subesophageal ganglion, and thoracic ganglia. In each of the three tissues, a large number of genes were differentially expressed, including genes encoding proteins involved with iron transport and homeostasis (e.g., transferrin, ferroportin, and metalloreductase STEAP3/4 homologs). The pulse treatment was also found to be linked to a number of other physiological processes, including functions associated with oxidative stress, immune response, DNA damage and repair, and mitochondria. Impacts on genes associated with these processes suggest potential deleterious effects of the pulse on the central nervous system.

This work is among the first to employ a transcriptomic approach to investigate the neural underpinnings of the magnetic sense, an active area of research in need of new, cutting-edge experimental techniques. Along with recent work in rainbow trout (Fitak et al., 2017; *in review*; Arniella et al., 2018), these studies provide a promising framework for exploring the genes and molecular pathways associated with magnetoreception. The finding that the expression of genes associated with iron regulation and oxidative stress is affected by

a magnetic pulse in both trout and lobsters provides molecular evidence consistent with iron-based magnetoreception in disparate species.

In addition, numerous genes linked to the crustacean immune response showed altered expression. It is possible that an immune response was induced by neural tissue damage resulting from the translocation of magnetite crystals or other iron-based structures within the nervous system. To explore this hypothesis, future work employing neurohistological techniques examining tissue damage and iron distributions in the nervous systems of pulsed and control animals is needed.

Interestingly, while the expression of genes associated with phototransduction was altered in all tissues examined, cryptochromes (light-sensitive proteins possibly involved with magnetoreception in some species; Liedvogel and Mouritsen, 2010) did not show changes in expression. While it has been hypothesized that a magnetic pulse should not have a lasting effect on the radical pairs mechanism of magnetoreception (Wiltschko et al., 2002), this has not yet been demonstrated. The work presented here only investigated gene expression at one time-point after exposure to the magnetic pulse treatment; future time-series studies will provide a more comprehensive picture of potential effects, if any, on cryptochromes and the radical pairs mechanism. In addition, further work confirming if and how phototransduction mechanisms are affected by a pulse in the lobster nervous system is clearly needed.

Finally, additional work is needed to investigate the functional significance of the observed expression patterns throughout the nervous system. Examining the responses of other tissues (e.g., muscle, heart, etc.) to a magnetic pulse is likely to elucidate general effects on biological tissue and might help to tease out effects that are specific to the nervous

system. In addition, future studies using qPCR to investigate the expression of the various candidate genes in a more specific manner (e.g., examining expression in the protocerebrum, deutocerebrum, and tritocerebrum of the brain separately) is needed. Immunohistochemistry techniques to more precisely localize and explore the distribution of the proteins produced by these candidate genes will also prove informative.

The results from this work significantly advance our knowledge of magnetoreception and provide novel insights into the effects of pulse magnetization on animal behavior and physiology. The apparent behavioral effects of a strong magnetic anomaly and a magnetic pulse provide support for magnetoreception in spiny lobsters and evidence that lobster magnetoreceptors are associated with permanently magnetic material. This is further bolstered by the finding that a pulse influences the expression of genes involved with iron regulation and the negative effects of free iron in the central nervous system. Nevertheless, it is clear that a magnetic pulse has a much more significant impact on neural physiology than previously assumed, emphasizing the limitations of using pulse magnetization as a diagnostic technique to exclusively affect magnetite-based magnetoreceptors.

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APPENDIX: CHAPTER V

Appendix 5.1: List of top significantly enriched GO terms in the brain. FDR=False discovery rate; BP=Biological Process; MF=Molecular Function; CC=Cellular Component.

GO Term	Description	*P*-value	FDR	Category
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	1.1×10^{-5}	0.0034	MF
GO:0003824	catalytic activity	1.1×10^{-5}	0.0034	MF
GO:0031224	intrinsic component of membrane	2.9×10^{-5}	0.0092	CC
GO:0016021	integral component of membrane	4.9×10^{-5}	0.0092	CC
GO:0016491	oxidoreductase activity	6.3×10^{-5}	0.01	MF
GO:0030246	carbohydrate binding	7.3×10^{-5}	0.01	MF
GO:0009055	electron carrier activity	0.0001	0.01	MF
GO:0016798	hydrolase activity, acting on glycosyl bonds	0.0001	0.01	MF
GO:0044710	single-organism metabolic process	1.2×10^{-5}	0.02	BP
GO:0004559	alpha-mannosidase activity	0.00037	0.028	MF
GO:0015923	mannosidase activity	0.00037	0.028	MF
GO:0044425	membrane part	0.00024	0.03	CC
GO:0016020	membrane	0.00034	0.032	CC

Appendix 5.2: List of top significantly enriched GO terms in the subesophageal ganglion. FDR=False discovery rate; BP=Biological Process; MF=Molecular Function; CC=Cellular Component.

GO Term	Description	*P*-value	FDR	Category
GO:0003735	structural constituent of ribosome	2.0×10^{-22}	1.2×10^{-19}	MF
GO:0005840	ribosome	1.1×10^{-20}	4.1×10^{-18}	CC
GO:0030529	intracellular ribonucleoprotein complex	4.8×10^{-15}	6.0×10^{-13}	CC
GO:1990904	ribonucleoprotein complex	4.8×10^{-15}	6.0×10^{-13}	CC
GO:0044391	ribosomal subunit	8.9×10^{-13}	8.3×10^{-11}	CC
GO:0005198	structural molecule activity	1.8×10^{-11}	5.5×10^{-9}	MF
GO:0006412	translation	4.1×10^{-11}	6.7×10^{-8}	BP
GO:0043043	peptide biosynthetic process	1.2×10^{-10}	6.7×10^{-8}	BP
GO:1901566	organonitrogen compound biosynthetic process	1.2×10^{-10}	6.7×10^{-8}	BP
GO:0043604	amide biosynthetic process	3.8×10^{-10}	1.6×10^{-7}	BP
GO:0015935	small ribosomal subunit	5.3×10^{-9}	4.0×10^{-7}	CC
GO:0006518	peptide metabolic process	1.6×10^{-9}	5.4×10^{-7}	BP
GO:1901564	organonitrogen compound metabolic process	2.3×10^{-9}	6.4×10^{-7}	BP
GO:0043603	cellular amide metabolic process	7.4×10^{-9}	1.8×10^{-6}	BP
GO:0022626	cytosolic ribosome	4.1×10^{-8}	2.6×10^{-6}	CC
GO:0044444	cytoplasmic part	8.2×10^{-8}	4.4×10^{-6}	CC
GO:0005737	cytoplasm	2.1×10^{-7}	9.8×10^{-6}	CC
GO:0022627	cytosolic small ribosomal subunit	1.7×10^{-6}	7.1×10^{-5}	CC
GO:0044445	cytosolic part	2.6×10^{-6}	9.8×10^{-5}	CC
GO:0019843	rRNA binding	1.9×10^{-6}	0.00039	MF
GO:0032991	macromolecular complex	1.6×10^{-5}	0.00055	CC
GO:0015934	large ribosomal subunit	1.9×10^{-5}	0.00059	CC
GO:0045263	proton-transporting ATP synthase complex, coupling factor F(o)	2.1×10^{-5}	0.00061	CC
GO:0005839	proteasome core complex	4.9×10^{-5}	0.0013	CC

GO:0009055	electron carrier activity	9.7×10^{-6}	0.0014	MF
GO:0004298	threonine-type endopeptidase activity	1.4×10^{-5}	0.0014	MF
GO:0070003	threonine-type peptidase activity	1.4×10^{-5}	0.0014	MF
GO:0043228	non-membrane-bounded organelle	6.4×10^{-5}	0.0015	CC
GO:0043232	intracellular non-membrane-bounded organelle	6.4×10^{-5}	0.0015	CC
GO:0044429	mitochondrial part	7.0×10^{-5}	0.0015	CC
GO:0015077	monovalent inorganic cation transmembrane transporter activity	3.7×10^{-5}	0.0032	MF
GO:0005743	mitochondrial inner membrane	0.00023	0.0048	CC
GO:0033177	proton-transporting two-sector ATPase complex, proton-transporting domain	0.00025	0.0049	CC
GO:0019866	organelle inner membrane	0.00028	0.0053	CC
GO:0019773	proteasome core complex, alpha-subunit complex	0.00035	0.0063	CC
GO:0005740	mitochondrial envelope	0.00042	0.0072	CC
GO:0031966	mitochondrial membrane	0.00049	0.0078	CC
GO:0005739	mitochondrion	0.0005	0.0078	CC
GO:0005215	transporter activity	0.00014	0.01	MF
GO:0022890	inorganic cation transmembrane transporter activity	0.00015	0.01	MF
GO:0000502	proteasome complex	0.00081	0.012	CC
GO:1905369	endopeptidase complex	0.00081	0.012	CC
GO:0022625	cytosolic large ribosomal subunit	0.00093	0.013	CC
GO:0016491	oxidoreductase activity	0.0004	0.024	MF
GO:0005829	cytosol	0.0019	0.026	CC
GO:0000313	organellar ribosome	0.0024	0.03	CC
GO:0005761	mitochondrial ribosome	0.0024	0.03	CC
GO:0022857	transmembrane transporter activity	0.00058	0.032	MF
GO:0009060	aerobic respiration	0.00019	0.035	BP
GO:0009142	nucleoside triphosphate biosynthetic process	0.00019	0.035	BP
GO:1905368	peptidase complex	0.0029	0.036	CC
GO:0045259	proton-transporting ATP synthase complex	0.0032	0.037	CC
GO:0015370	solute:sodium symporter activity	0.00075	0.038	MF
GO:0006099	tricarboxylic acid cycle	0.00029	0.041	BP

GO:0009145	purine nucleoside triphosphate biosynthetic process	0.00029	0.041	BP
GO:0009206	purine ribonucleoside triphosphate biosynthetic process	0.00029	0.041	BP
GO:0022892	substrate-specific transporter activity	0.001	0.047	MF

Appendix 5.3: List of top significantly enriched GO terms in the thoracic ganglia. FDR=False discovery rate; BP=Biological Process; MF=Molecular Function; CC=Cellular Component.

GO Term	Description	*P*-value	FDR	Category
GO:0005975	carbohydrate metabolic process	5.1×10^{-6}	0.0086	BP
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	1.7×10^{-5}	0.01	MF
GO:0016798	hydrolase activity, acting on glycosyl bonds	3.7×10^{-5}	0.011	MF
GO:0030246	carbohydrate binding	0.0001	0.018	MF
GO:0008483	transaminase activity	0.00015	0.018	MF
GO:0016769	transferase activity, transferring nitrogenous groups	0.00015	0.018	MF

Appendix 5.4: Ranked list of enriched GO terms in response to the magnetic pulse treatment. Average rank and score are calculated means for all genes assigned to each GO term, indicating the power to differentiate between the pulsed and control groups. Count=number of genes assigned to GO term; BP=Biological Process; MF=Molecular Function; CC=Cellular Component.

GO Term	Average Rank	Average Score ($\times 10^3$)	Count	*P*-value	Description	Category
GO:0000028	2471.67	0.8	6	0.011	ribosomal small subunit assembly	BP
GO:0006006	2497.6	1.95	5	0.014	glucose metabolic process	BP
GO:0006816	2511.67	4.28	6	0.009	calcium ion transport	BP
GO:0004888	2642.56	2.2	9	0	transmembrane signaling receptor activity	MF
GO:0003756	2678.4	1.19	5	0.022	protein disulfide isomerase activity	MF
GO:0006890	2685.6	1.2	5	0.015	retrograde vesicle-mediated transport, Golgi to ER	BP
GO:0006635	2691.8	1.34	5	0.027	fatty acid beta-oxidation	BP
GO:0007030	2726.4	2	5	0.02	Golgi organization	BP
GO:0005891	2743	0.98	6	0.015	voltage-gated calcium channel complex	CC
GO:0006465	2788.8	5.17	5	0.025	signal peptide processing	BP
GO:0050789	2819	1.24	8	0.008	regulation of biological process	BP
GO:0071897	2830.88	0.99	8	0.006	DNA biosynthetic process	BP
GO:0046835	2846.22	6.69	9	0.011	carbohydrate phosphorylation	BP
GO:0035999	2886.5	1.69	6	0.03	tetrahydrofolate interconversion	BP
GO:0031982	2888.2	0.51	5	0.04	vesicle	CC
GO:0003713	2896.38	0.96	16	0	transcription coactivator activity	MF
GO:0006544	2897.8	1.42	5	0.039	glycine metabolic process	BP
GO:0045859	2919.2	0.32	5	0.048	regulation of protein kinase activity	BP
GO:0030414	2925.17	13.22	12	0.007	peptidase inhibitor activity	MF
GO:0002098	2942.71	2.03	7	0.025	tRNA wobble uridine modification	BP
GO:0004003	2959	4.88	7	0.017	ATP-dependent DNA helicase activity	MF
GO:0006265	2967.8	0.84	5	0.038	DNA topological change	BP
GO:0003714	2979.5	1.53	6	0.029	transcription corepressor activity	MF

GO:0031981	3037.62	0.64	13	0.005	nuclear lumen	CC
GO:0004497	3039.31	0.81	13	0.007	monooxygenase activity	MF
GO:0032947	3040.6	0.64	5	0.046	protein complex scaffold	MF
GO:0006974	3042.3	2.24	10	0.007	cellular response to DNA damage stimulus	BP
GO:0006303	3065	4.44	8	0.036	double-strand break repair via nonhomologous end joining	BP
GO:0015914	3074	2.17	6	0.044	phospholipid transport	BP
GO:0015035	3074.36	1.95	11	0.013	protein disulfide oxidoreductase activity	MF
GO:0016746	3089.75	0.77	20	0	transferase activity, transferring acyl groups	MF
GO:0051276	3094.44	1.1	9	0.019	chromosome organization	BP
GO:0005245	3104.67	0.65	6	0.047	voltage-gated calcium channel activity	MF
GO:0044767	3128.57	3.56	7	0.05	single-organism developmental process	BP
GO:0048731	3202.17	5.62	6	0.045	system development	BP
GO:0042802	3204.38	3.23	16	0.005	identical protein binding	MF
GO:0005694	3239.6	2.60	10	0.023	chromosome	CC
GO:0019901	3245.36	0.57	11	0.018	protein kinase binding	MF
GO:0005623	3258.88	3.75	16	0.011	cell	CC
GO:0048513	3267.38	1.03	8	0.044	animal organ development	BP
GO:0034765	3278.78	0.56	9	0.043	regulation of ion transmembrane transport	BP
GO:0006289	3284.42	0.51	12	0.011	nucleotide-excision repair	BP
GO:0006302	3349.8	0.54	10	0.037	double-strand break repair	BP
GO:0009055	3358.29	1.15	31	0.001	electron carrier activity	MF
GO:0004601	3375.69	0.38	13	0.027	peroxidase activity	MF
GO:0008033	3390.37	1.47	19	0.017	tRNA processing	BP
GO:0003950	3423.25	0.75	12	0.038	NAD+ ADP-ribosyltransferase activity	MF
GO:0005730	3457.19	1.27	47	0.001	nucleolus	CC
GO:0004252	3478.71	0.92	77	0	serine-type endopeptidase activity	MF
GO:0005102	3515.43	0.85	14	0.041	receptor binding	MF
GO:0043227	3524.79	1.58	19	0.038	membrane-bounded organelle	CC
GO:0006310	3567.13	4.82	15	0.048	DNA recombination	BP

GO:0006030	3617.86	3.02	28	0.025	chitin metabolic process	BP
GO:0043231	3650.62	0.89	34	0.019	intracellular membrane-bounded organelle	CC
GO:0020037	3696.61	0.65	49	0.022	heme binding	MF
GO:0006260	3701.7	2.21	33	0.035	DNA replication	BP
GO:0030529	3789.33	0.71	39	0.039	intracellular ribonucleoprotein complex	CC
GO:0005576	3828.05	2.82	93	0.008	extracellular region	CC
GO:0006412	3833.11	0.52	122	0.002	translation	BP
GO:0006351	3853.88	0.85	58	0.043	transcription, DNA-templated	BP
GO:0008168	3854	0.93	57	0.034	methyltransferase activity	MF
GO:0003824	3887.51	0.47	143	0.006	catalytic activity	MF
GO:0005737	3922.38	1.1	368	0	cytoplasm	CC
GO:0016787	3958.51	0.97	111	0.038	hydrolase activity	MF
GO:0003700	3975.21	0.60	148	0.034	transcription factor activity, sequence-specific DNA binding	MF
GO:0003735	3980.25	0.47	132	0.041	structural constituent of ribosome	MF
GO:0006508	3983.58	0.87	222	0.021	proteolysis	BP
GO:0055114	3988.9	0.77	323	0	oxidation-reduction process	BP
GO:0005634	4050.17	0.67	496	0.008	nucleus	CC